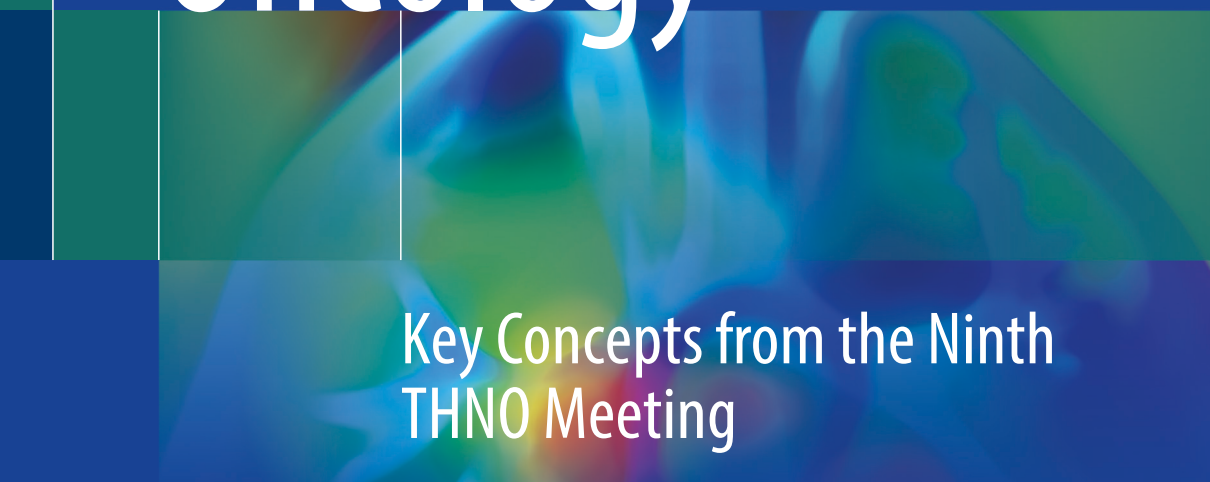
# Critical Issues in Head and Neck Oncology



## Key Concepts from the Ninth THNO Meeting

Jan B. Vermorken
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*Editors*

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# Preface THNO-9

The ninth Trends in Head and Neck Oncology (THNO-9) took place at the NH-Hotel Málaga in Málaga, Spain, November 9–11, 2023. It was coordinated by the same organizing team as on the last four occasions with support from Pharma (Merck, Nanobiotix, and MSD) and practical logistical support from Congress Care. The conference was also endorsed by the European Head and Neck Society (EHNS), the European Organization for Research and Treatment of Cancer (EORTC), the European Society for Radiotherapy and Oncology (ESTRO), and the "Grupo Español para el Tratamiento de Tumores de Cabeza y Cuello (TTCC)." As on previous occasions, the format was educational, with a multidisciplinary focus but also with emphasis on cutting-edge research, outcome data, and clinical application. Case presentations, organized by some members of the coordinating team and some local doctors, stimulated lively interaction between faculty and audience and stressed the importance of individualized patient care underpinned by the best available evidence. Thanks to the dedication of the faculty members this book will be available soon after the actual meeting, with manuscripts prepared in the first half of 2024, guaranteeing the most up-to-date information in this rapidly evolving field. We are most grateful to them for their efforts in realizing this important goal. Special thanks goes to Dr. Petr Szturz, who helped in the review process for the different manuscripts.


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**Leon J. Wils and Ruud H. Brakenhoff**

## Oral Potentially Malignant Disorders

While most head and neck squamous cell carcinomas (HNSCC) arise *de novo*, in some cases they are preceded by clinically visible lesions defined as oral potentially malignant disorders (OPMD). OPMDs are defined as 'any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer' [1]. OPMDs may present with a variety of clinical aspects and mostly as a white or red patch in the mucosal epithelium. However, not every white lesion is an OPMD. Frictional lesions caused by the teeth against the mucosal lining may present as white changes, but these lesions are not associated with increased risk for cancer and consequently are not classified as OPMDs. Per definition, OPMDs are distinct from carcinomas and do not have any invasive capabilities and lack the ability to metastasize [2]. They might undergo malignant transformation (MT), and the risk depends on many variables including the specific type of OPMD. Histologically, MT follows a number of steps from epithelial hyperplasia to dysplasia, divided into three grades (mild, moderate and severe) and eventually invasive carcinoma. More recently architectural dysplasia has been identified as a separate pattern of dysplasia [3–5].

The term OPMD was introduced in 2007 and is currently incorporated in the latest classification of head and neck tumors by the World Health Organization (WHO) [6, 7]. The overall worldwide prevalence for OPMDs is estimated at 5%, with higher frequencies in Asians and males [8]. As OPMDs may precede oral squamous cell carcinoma (OSCC), risk factors are similar and include (smokeless) tobacco,

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alcohol and betel nut use [9]. In addition, some of the OPMDs may be present next to OSCC [1]. OPMDs may present with a wide range of clinical features including color variations, topographic changes and size [10, 11]. OPMDs may affect all anatomical sites in the oral cavity and may be present at one or more multiple subsites simultaneously [12].

## Oral Leukoplakia

### *Epidemiology*

Oral leukoplakia (OL) is the most common OPMD and is defined by the WHO as "a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" [1, 6, 7]. Incidence data for OL are scarce and only reported for specific Asian regions ranging from 0.1% to 0.2% [13, 14]. The pooled prevalence in the general population was estimated to be between 1.49% and 4.11% based on more than 1000 individuals [8, 15], but given the incidence of oral cancer these figures seem biased. OL has a reported annual MT rate of 1% to 10%. Consequently, patients have a 30% to 50% lifetime risk of developing OSCC [15–18]. While demographics for the general population have been published, these values may substantially differ between geographical locations due to the presence of different risk factors [8, 16]. Hence, reliable figures are difficult to obtain and vary per region, but assuming that ~30% of oral cancers is preceded by OL (28% at our center), a median time to transformation of 7 years and a life-time risk of 50% for MT, the prevalence of OL should theoretically be approximately 5 times higher than the incidence of oral cancer.

### *Clinical Management*

OL presents as a white patch or plaque that cannot be scraped off. OL is a clinical diagnosis having excluded other recognizable white or white/red lesions. Based on the clinical presentation, OL is classified as a homogeneous (predominantly white, flat, thin or wrinkled) or non-homogeneous (mixed white-and-red, including speckled, nodular, granular or verrucous) lesion. Before a definitive histopathological diagnosis of OL can be made, potential other factors that may cause lesions such as (smokeless) tobacco use, friction (mechanical irritation) or contact (dental restorations) should be omitted. If the lesion does not disappear after a certain observation period, a biopsy is performed [1, 12]. Small lesions are generally excised while for larger lesions an incisional biopsy is performed. The specimen is examined under the microscope to exclude the presence of invasive carcinoma and to determine the absence or presence and grade of dysplasia, morphologically abnormal presentations of the mucosal epithelium. When invasive carcinoma is excluded by histology

and following an observational period that may vary between three months and one year to exclude micro-invasive carcinomas that might have been missed in the biopsy, the definitive diagnosis of OL is made. Despite the policy to excise lesions when possible, there is no evidence at present that treatment of OL effectively prevents the development of oral cancer [16, 19]. Therefore, all patients remain under frequent and, if possible, lifelong surveillance at oral medicine and head and neck cancer centers. There is a need for better risk stratification of OL lesions for the prediction of MT of these lesions as this may reduce burden on the patient and the healthcare system for frequent lifelong surveillance. Risk stratification may be used to select patients for either less intensive monitoring, regular follow-up by their dentist or enrollment in experimental trials using novel treatments.

## *Risk Assessment of Oral Leukoplakia*

Several clinical characteristics have been investigated in relation to the risk of MT of OL. Presence of OL is clearly associated with the use of (smokeless) tobacco, alcohol and betel nut, but the precise attributable fraction of these exogenous carcinogens is unknown, also because many studies report missing data [11, 12, 16, 20, 21]. In contrast, the relation between risk for MT and exposure to these exogenous carcinogens has not been established. Betel nut use seems to be associated with a higher risk for MT, but this correlation is not consistently reported [20, 22, 23]. With regard to tobacco use, the risk of MT of OL seems to be even higher for non-smokers compared to smokers, a somewhat counterintuitive observation [17, 20, 22–28]. There is no established correlation between use of alcohol and risk for MT [17, 20, 23, 25, 28].

The clinical presentation of OL with respect to location, homogeneity and size may further influence the risk of MT. However, this might in part again be related to the etiological factors, and consequently the geographic location, as some risk factors such as betel nut use are region-associated. Likewise, ethnicity and cultural risk factors influence the location of OL as certain habits are associated with the presence of OL at specific subsites [8]. For example in populations from developed countries, OL is most frequently found at the lateral tongue and floor of mouth [12, 25–29]. In contrast, in Asian countries the gingivobuccal region is often affected due to placement of betel nut and smokeless tobacco [29–31]. The most common clinical type of OL reported is homogeneous OL, but a higher rate of MT is reported for non-homogeneous OL [11, 32, 33]. Moreover, lesions larger than 200 $mm^2$ have a higher risk of MT, compared to smaller lesions [11, 28, 32, 33].

Other clinical features that may correlate with risk of MT are age and gender. OL generally presents in male patients after the fourth decade of life at a median age of around 60 years [16, 29, 32]. Based on a recent systematic review that included some older studies, age appeared to correlate with MT, and older patients seemed at higher risk [32]. In addition, while OL is more common in males, females are at a higher risk of MT [32]. More recently, similar studies were reported but effects of

age and gender on MT were not apparent in all cohorts [17, 20, 31]. However, comparing clinical cohorts remains problematic because of diagnostic definitions and confounding etiological factors.

Fanconi anemia (FA) is a hereditable syndrome characterized by congenital abnormalities, progressing bone marrow failure and cancer predisposition. The disease is caused by a mutation in one of the genes involved in DNA crosslink repair. FA patients have a substantially increased risk of HNSCC, and they also have an increased risk of acquiring OL, with a reported prevalence of 4% to 18%, regardless of whether patients receive a stem cell transplant to treat their bone marrow failure or not [34–37]. Robust data regarding the risk of MT of OL in FA patients is lacking, as these data are only reported sporadically [36, 37], but given the high incidence of oral cancer in these patients, the risk for MT is likely increased.

## *Morphology*

As indicated above, in addition to the clinical examination, a biopsy is taken and examined by microscopy; first to exclude the presence of invasive carcinoma, and second to determine the presence or absence of morphological changes of the epithelium coined as dysplasia. Presence of dysplasia is an important risk factor for MT of OL [32]. The WHO employs a three-tiered grading system for oral epithelial dysplasia: mild, moderate and severe [6, 38]. To adhere to the most recent WHO guidelines the term 'cytological dysplasia' is utilized for this type of morphological change that impacts the cellular morphology [3, 6, 39]. Cytological dysplasia is present in 19% to 70% of OL lesions [17, 32, 40]. This large discrepancy relates to the different definitions employed for the diagnosis of OL, and the difficulties to recognize the milder forms of dysplasia. OL with presence of dysplasia is generally at an increased risk for MT, and this risk increases with dysplasia grade. However, this relationship is not always evident and lesions without cytological dysplasia may also transform into OSCC [17, 31, 32]. In addition to cytological dysplasia, the WHO updated the definitions of dysplasia in 2022, and added a description of architectural features without pronounced cytological atypia that may in themselves signify dysplasia (Fig. 1.1) [6, 41]. This pattern was initially identified as 'differentiated dysplasia' in the vulva epithelium and thereafter tested for diagnosis and risk assessment of OL [3–5, 42]. The added value of this architectural form of dysplasia improved risk assessment tremendously. Lesions that were diagnosed histologically as no apparent classic cytological dysplasia could be stratified for progression risk by architectural dysplasia [3, 4]. Thus far, this was based on several single center studies, and a large multicenter study is awaited to confirm these results.

Although these new data are very promising, the application of classic cytological dysplasia only as a marker for the prediction of MT remains imperfect by lack of reproducibility [6, 41, 43]. While several refined updates in grading have been proposed, the inter-observer agreement still varies widely from only slight agreement ($\kappa = 0.22$) to substantial agreement ($\kappa = 0.78$) [4, 40, 44–47]. These

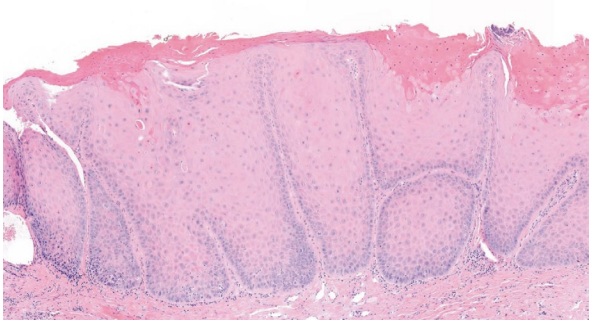

**Fig. 1.1** Example of the architectural pattern of dysplasia. Besides the classic epithelial dysplasia graded as mild, moderate and severe, also so called 'architectural dysplasia' is recognized and shown to be clinically very relevant for risk assessment of leukoplakia. In classic epithelial dysplasia the tissue shows enlarged and hyperchromatic nuclei, decreased nuclear–cytoplasmic ratio, mitoses in suprabasal layers and loss of differentiation towards the surface. As depicted in the haematoxylin-eosin staining, architectural dysplasia is characterized by a basal layer of small cells with hyperchromatic or open nuclei with small nucleoli, and it shows an abrupt transition to suprabasal large cells with abundant, eosinophilic cytoplasm and large, open nuclei with prominent nucleoli. (Example obtained from Wils et al. Clin Cancer Res 2023)

differences may relate to lack of standardization and subjectivity, and are particularly prominent for recognizing and grading of milder forms of dysplasia [6, 41, 44]. In some studies, a binary system for the prediction of MT is employed by which lesions are divided into low risk (no or mild cytological dysplasia) or high risk (moderate or severe cytological dysplasia). This system is associated with a higher inter-observer agreement, but whether it enables a better prediction of MT compared to the standard WHO classification remains inconclusive [4, 41, 44, 45, 47].

A variety of immunohistochemical biomarkers have also been exploited. Both cytokeratin 13 and cytokeratin 17 are two histological markers used for the identification of OL lesions at risk for MT, in that dysplastic lesions show a decreased expression of cytokeratin 13 and an increased expression of cytokeratin 17 [3–5, 48, 49]. This switch in expression is associated with higher grades of dysplasia and an increased risk of MT of OL [4, 5, 48, 50, 51]. These biomarkers do not seem to outperform routine histopathology, but are a great aid to recognize abnormal mucosal epithelium when morphological changes are more subtle such as in the case of architectural dysplasia [3–5].

## *Genomic Landscape*

Cancer is caused by an accumulation of genetic and epigenetic changes, and consequently genetic markers may serve well as biomarkers for MT prediction. Exploration of the genomic landscape of OL has revealed various genetic biomarkers that can be utilized for the prediction of MT of OL. As OL potentially precedes

OSCC, observed genetic changes in leukoplakia are often also occurring in OSCC. Since the 1990s the genetic landscape of OL has been extensively studied. Copy number alterations (CNA) are present in 12% to 93% of OL lesions [30, 52–60]. The large variation in frequencies may be attributed to the techniques used for the detection of genetic changes. Initially, the chromosomal aberrations in OL were investigated by loss of heterozygosity (LOH) analysis using microsatellite PCR [54]. Based on LOH, 9p loss, 3p loss, 13q loss and 17p13 loss were identified to be most frequently affected regions in dysplastic oral lesions [54, 61]. Additional changes were identified later in progression, such as 4q loss, 8p loss and 11p loss [52, 62]. LOH of 3p14 and 9p21 seemed to be the most informative markers to predict MT of OL [52, 55]. Obviously, this was established before the recognition of architectural dysplasia as a morphological biomarker for predicting MT. DNA ploidy, a more general marker for chromosomal changes that is measured by fluorescence in situ hybridization, fluorescence activated cell sorting or cytometry, is often present in OL, but lacks the specificity of LOH and this marker is not very suitable for prediction of MT of OL [56, 57, 63]. Next generation sequencing (NGS) allows for the simultaneous detection of genome wide CNAs, focal aberrations, as well as mutations, and the entire genome may be deep sequenced at once. So far NGS has only been employed sporadically for the detection of genetic changes in OL. Two studies reported at least one CNA in 65% to 72% of cases and identified additional frequently affected regions, for example gain of 20q [30, 60].

While chromosomal instability in OL has been investigated for decades, mutations have only been investigated since the introduction of NGS and often only in limited or very specific data sets. In one study only *NOTCH1* was investigated, and mutations were reported in 60% of OL samples [64]. In other studies, whole exome sequencing (WES) was employed to identify mutations in multiple genes. These studies most often reported mutations in known OSCC driver genes, for example: *TP53* (14% to 38%), *CDKN2A* (11% to 23%), *CASP8* (30% to 46%), *NOTCH1* (21%) and *FAT1* (14%) [30, 65–67]. While most driver genes are mutated in comparable frequencies between OL and HNSCC, *TP53* is mutated significantly less in OL. In contrast, OL seems to harbor more *CASP8* mutations compared to OSCC. However, *CASP8* mutations were only reported for a cohort of patients with OL lesions that were present adjacent to tumors in the gingivobuccal region and might therefore not be representative for patients with OL in general [30, 65]. In addition, studies reported mutations in genes responsible for DNA damage repair or upregulation of genes that may cause DNA damage [30, 65, 66]. These mutations may contribute to the increasing chromosomal instability and number of mutations found in OL and OSCC. Recently, a study was published exploiting NGS in the context of architectural dysplasia risk assessment. It was shown that absence of any dysplasia, both classic cytological dysplasia and architectural dysplasia, was associated with an extremely low risk for MT. However, the cancer risk for dysplastic lesions was still a mere 50%, which could be further stratified by genetic markers. This led to a three-tiered risk model: OL lesions were first stratified based on presence of dysplasia where lesions without any dysplasia were defined as low risk, and

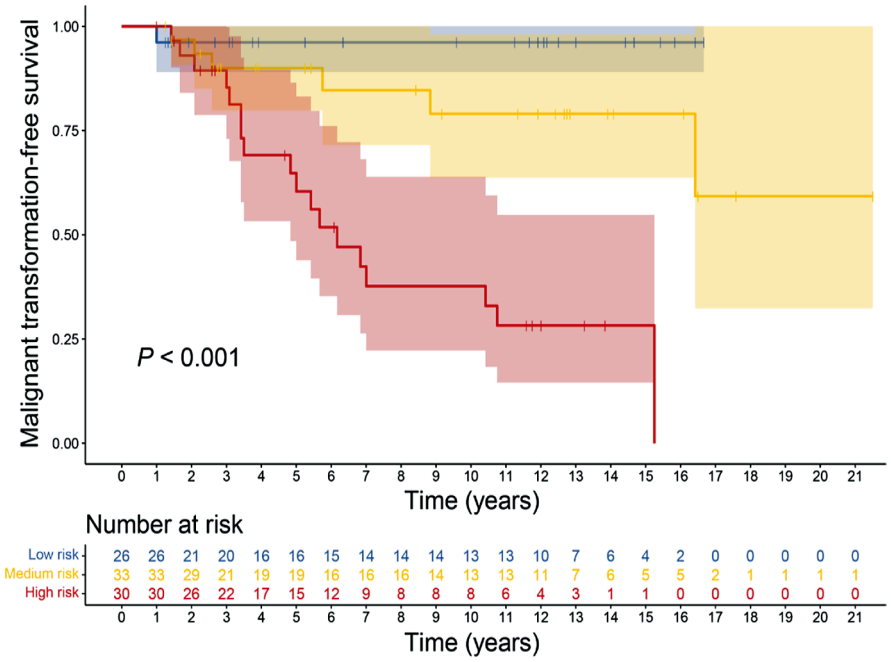


**Fig. 1.2** Kaplan–Meier curves for malignant transformation-free survival of three stratified groups as defined by the presence of dysplasia and selected genetic changes. On the x-axis the time in years from the date of first biopsy. The high-risk group had a significantly higher risk of malignant transformation compared with the medium risk group (HR, 4.99; 95% CI, 1.83–13.64; P = 0.002). (Obtained from Wils et al. Clin Cancer Res 2023)

those with dysplasia were further stratified as medium or high risk based on genetic factors (Fig. 1.2) [39].

Besides these genetic changes as biomarkers, gene expression profiling has been used to predict MT of OL. Already a decade ago it was shown that it was possible to stratify OL lesions for cancer risk based on RNA expression profiles [68].

## Prevention of Malignant Transformation of Oral Leukoplakia

When treatment is feasible, surgical excision and CO2 laser evaporation are used most frequently for the management of OL. However, despite treatment, up to 49% of OL lesions recur within 5 years after treatment, and surgical excision of the OL lesion does not prevent MT [69]. It should be noted, however, that data regarding the efficacy of surgery are obtained from observational studies, which are obviously biased, and not from randomized controlled trials (RCT) [70]. There is currently an RCT in progress (NCT04858100), in which surgical excision is compared to a 'wait and see policy', although it could be better indicated as a watchful surveillance

policy. In this RCT, oral cancer prevention is the primary endpoint. In total 310 patients will be enrolled, and completion is expected in 2026. Besides excision, several other interventions are being applied, some of which lack clinical evidence, some that have been tested in the past and some that are currently being evaluated in clinical trials [16, 19]. These treatments can roughly be divided into the following categories: surgery, chemical compounds, immune checkpoint inhibitors and natural compounds.

## *Surgery*

In addition to 'cold knife' excision, multiple other surgical methods have been tested. Electrocauterization or electrocoagulation has been employed in the distant past, but this induction of thermal damage causes pain, edema and scarring of the underlying tissues [71, 72]. Cryosurgery is an easy to apply therapeutic method that uses freezing to destruct locally the target tissue. Most often liquid nitrogen was used which may be applied directly or through spray [73–75]. Although in some studies complete removal of lesions in all patients has been reported after cryosurgery, lesions often recurred and cryosurgery was associated with postoperative pain and edema [72, 74–77]. Moreover, epithelial regeneration may even promote tumor development as is evident from an increased frequency of MT in OL treated with cryosurgery [78, 79].

Laser surgery is supposed to be associated with several advantages compared to cold knife surgery, such as better control of bleeding, accurate lesion removal, minimal adjacent tissue damage, reduced post-operative pain, less edema and infection and a lower recurrence rate of OL [72, 80]. Laser evaporation has been in use for the treatment of OL since the 1970s [81]. At least six types of lasers have been tested for the treatment of OL, of which the CO2 laser is most often used [72, 82]. The various laser types differ in the specific wavelength they emit, which in turn determines the type of application they are most suitable for. In addition, lasers may be used to activate photosensitizers administered to the lesion, for photodynamic treatment [83, 84]. In two systematic reviews discussing some or all of the different laser types it was found that recurrence rates were similar and that, independent of the type of laser, MT occurred in 5.2% of the patients. Patients that received these treatments therefore need to remain in follow-up. In addition, there is a lack of prospective RCTs, and therefore results are difficult to compare [80, 84, 85].

## *Chemical Compounds*

Particularly in case of OL, chemopreventive medication has been used for the treatment of the lesion or alternatively suppression of progression of the lesion to OSCC. Many chemical compounds, targeting a plethora of cellular pathways and

processes, have been investigated in observational studies and clinical trials to prevent oral cancer.

The deregulation of the cell cycle plays an important role in OL initiation and progression, as is evident from the high frequency of mutations in *TP53* and *CDKN2A*. However, these mutations typically inactivate tumor suppressor proteins and are therefore not directly druggable. These mutations cause unscheduled S-phase progression with associated replication stress, which might offer the opportunity for the exploitation of synthetic lethality by drug or gene interactions [86]. Bleomycin might be an example in this respect as it induces double strand breaks which in combination with *TP53* mutations result in cell death. Bleomycin has been tested clinically and caused disappearance of lesions, but most patients reported side effects and there was an increased risk for MT [16, 87, 88]. More recently, genomic screens in precancer cell lines have revealed more specific therapeutic targets, such as *PLK1* and *WEE1* [89–91]. There are several small molecule inhibitors that target these proteins, which might be a promising strategy to follow. However, also these inhibitors show toxic side effects in studies in cancer patients and need to be tested for treatment of precancerous lesions in clinical trials.

Targeting activated oncogenes may be a second viable option in prevention of MT of OL, but the number of activated oncogenes in HNSCC is rather scarce. The epidermal growth factor receptor (*EGFR*) might be a candidate as it is upregulated in OL and further influences HNSCC driver genes *PIK3CA* and *HRAS*. Multiple EGFR inhibitors have been investigated in clinical trials, but with very limited success. There were no effects of erlotinib and cetuximab on reducing the MT rate of OL, but the follow-up period in these studies was relatively short and sample sizes relatively small. Vandetanib, a wide spectrum kinase inhibitor targeting VEGFR, the RET-oncogene and EGFR has been tested as well and is currently tested in a clinical trial. Overall, while targeting EGFR seems to have some biological activity, there is currently no evidence that it inhibits MT of OL [62, 92–94].

Several drugs that are commonly used to treat other diseases may also have potential in the treatment of OL. Already for many years it has been reported that OSCC incidence is decreased in type II diabetes patients, suggesting that anti-diabetic drugs that decrease glucose levels in these patients might have protective effects [95]. While associations are not necessarily causative (obese patients at risk for diabetes type II might also be more frequent alcohol consumers and smokers), these drugs may be effective, and in multiple clinical trials the potential of metformin and pioglitazone to prevent MT of OL is currently being evaluated. Initial results seem promising but final results have not yet been reported [96–99]. A totally other class of drugs that have been tested are the non-steroidal anti-inflammatory drugs (NSAID), such as celecoxib, aspirin and ketorolac. Their efficacy in treatment of OL has been assessed in clinical trials but none were effective in preventing MT, while severe adverse side effects were reported [94, 100–103]. Finally, sodium valproate, an anti-epileptic drug, is currently in clinical trial.

## *Immune Checkpoint Inhibitors*

More recently, the immune system emerged as a potentially important player in the MT of OL. HNSCC is associated with an immunosuppressive microenvironment, and it has been hypothesized that these changes occur during the pre-malignant phase [104–106]. Increased PD-L1 expression on epithelial cells is associated with an increased risk of MT, which opens up the rationale for the use of immune checkpoint inhibitors that are already successfully used in the treatment of HNSCC [106, 107]. There are currently clinical trials underway assessing the efficacy of pembrolizumab, nivolumab and avelumab. Patients are selected based on the presence of high-risk changes, usually 3p and 9p LOH, and studies assess the efficacy to prevent the MT of OL. Recently results with nivolumab have been reported. For this study, a total of 33 patients were included in a nonrandomized, open-label trial, with a particularly aggressive subtype of OL, proliferative OL, which is characterized by heterogeneous or verrucous lesions, often involving multiple oral subsites. Patients received four doses of nivolumab every 28 days, and subsequently followed for up to 48 months. After the initial treatment period, a response was observed in 12 patients, while progressive disease was observed in four patients. Nine lesions developed into a malignancy, six of which initially showed a response to the treatment. While the study reached its prespecified primary endpoint, the change in size and degree of dysplasia, and the overall response with respect to cancer prevention was relatively disappointing. In addition, two patients withdrew from the study due to toxic effects, and seven patients experienced grade 3 or 4 adverse effects. Study follow-up is still underway, and additional results may be presented in the coming years. Results for other immune checkpoint inhibitors are expected in the coming years.

## *Natural Compounds*

Natural compounds have long been implicated in the treatment of OL. Vitamin A, its precursors such as beta-carotene and its retinoid derivatives are important for many essential biological processes, including normal development of epithelial cells. In turn, vitamin A deficiency is associated with an increased risk for OL and OSCC [108, 109]. Therefore, several studies have employed beta carotene, vitamin A and retinoids as treatment for OL [16, 88, 109]. However, their efficacy has never been convincingly proven and toxic side effects were frequently reported. Other natural compounds that have been assessed as a treatment for OL are lycopene, curcumin and caffeine [16, 110–114]. While these compounds have been investigated in multiple clinical trials as a treatment for OL, this was mostly in monocenter studies and when older definitions for OL were used, and the results were again not convincing. Randomized clinical studies with long follow-up and cancer development as a primary endpoint will be required to show clinical value of these compounds.

### *Oral Leukoplakia Cell Models*

There are currently no treatments that prevent MT of OL. However, multiple promising druggable target genes for treatment have been identified. Small molecule inhibitors targeting these genes need to be validated *in vitro* and *in vivo* before they can be applied as treatment in OL patients. To allow this, cell models would be a great aid. Several *in vitro* OL cell models have been generated in the past decades, but only a few successful culture models have been reported. Initially, cell lines were obtained by culturing biopsies obtained from OL biopsies on 3T3 fibroblast feeder layers. Some cultures proliferated for a limited number of passages while others seemed immortal. An extended period of proliferation is a necessity to apply treatment assays [115–118]. More recently, oral keratinocytes were cultured from tumor-adjacent mucosa [119]. Interestingly, all immortal cell cultures lost expression of *TP53* or *CDKN2A* or both, suggesting their importance for *in vitro* oral keratinocyte culture [115–119]. When tested, these cultured OL and tumor-adjacent cells did not display any take rate in mice, confirming their pre-malignant nature. While many of these cultures display an extended lifespan as a high number of population doublings can be achieved, cells may still cease proliferation due to replicative senescence caused by telomere shortening [120]. Telomerase may be activated in these cells to overcome replicative senescence [121]. Nowadays, CRISPR/Cas9 enables researchers to selectively knock-out genes. This technique may be employed to create OL cell lines that can grow independently of supporting cell layers [121, 122]. A critical question, of course, is whether these engineered precancer cell lines really represent the *in vivo* lesions to allow clinical translation of findings. However, the lack of an effective treatment hampers the field tremendously and also impacts the research on risk assessment modeling. Risk stratification of OL patients is particularly useful when treatments could be applied.

## Conclusions

OL is the most common oral potentially malignant disorder with a high risk of MT to OSCC. In recent years, major steps have been made in consensus definitions, and it is essential that researchers in the field follow these. In current literature there is a wide range in reported annual malignant transformation rate. This range may be attributed to different etiological factors, definitions used for the diagnosis of OL and the length of follow-up. Recent identification of new morphological and genetic biomarkers resulted in better risk stratification of patients with OL to predict MT which in the future may reduce the burden on patients and oncology centers and enable selection of patients for experimental trials. There is currently no evidence of any effective treatment of OL preventing recurrence of the lesion or MT, and all patients should preferably be kept under frequent watchful surveillance for a long

time or even lifelong with the goal to detect MT at an early stage. Development of novel treatments is essential to improve the management of OL and OPMD, and in a broader sense, to prevent OSCC.

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# Chapter 2
# An Introduction to Artificial Intelligence in Medicine and Its Role in Oral Potentially Malignant Disorders (OPMD)


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## Introduction

Precancerous lesions were first described in 1805 by a European panel of experts, who defined them as benign conditions that may develop over time into invasive malignancies [1]. This concept was further refined specifically for the oral cavity in 2005 at a workshop coordinated by the WHO Collaborating Centre for Oral Cancer and Precancer. Here, experts proposed a group of lesions and disorders that may be associated with an increased risk of oral squamous cell carcinoma (OSCC). These associations were based on several factors: alterations that co-exist with OSCC, alterations that share similar cellular changes, and the detection of chromosomal, genomic, and molecular alterations present in both precancerous lesions and OSCC [2]. These lesions were termed Oral Potential Malignant Disorders (OPMD) and

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**Table 2.1** OPMDs considered by the WHO workshops in 2005 and 2020

| **Oral potential malignant disorders (OPMD)** |
|---|
| Leukoplakia |
| Erythroplakia |
| Palatal lesions in reverse smokers |
| Oral submucous fibrosis |
| Actinic keratosis |
| Lichen planus |
| Discoid lupus erythematosus |
| Hereditary disorders with increased risk |
| **Extra conditions added in 2020** |
| Oral lichenoid lesions |
| Oral graft versus host disease |

described as various mucosal disorders that may precede the diagnosis of OSCC [3]. A total of nine lesions were classified as OPMDs at the first workshop in 2005 [1], with two additional conditions added at a second workshop held in 2020 [4] (as detailed in Table 2.1).

The importance of studying OPMDs has recently been highlighted in the Handbook of Oral Cancer Prevention published by the International Agency for Research on Cancer [5]. Interest in OPMDs has increased due to the possibility of understanding the many factors and biological events leading to the development of OSCC and developing strategies to intercept malignant transformation [6]. Several papers have investigated the etiology of OPMD, showing a strong association with tobacco [7] and alcohol [8] habits, together with betel quid chewing in Southeast Asia and the Pacific Islands [9], micronutrient deficiencies [10] and, to a lesser extent, human papillomavirus infection [11], among others, being the most frequently cited contributing factors [12].

Despite significant research efforts, many challenges remain in the management of patients with OPMD, ranging from screening, diagnosis, treatment, risk stratification and patient follow-up, posing significant hurdles for both patients and healthcare providers. Visual inspection and palpation remain the standard for **screening**. *In vivo* optical imaging methods have been proposed to increase the sensitivity of OPMD detection, *i.e.* autofluorescence imaging, narrow band imaging and Raman spectroscopy. Their advantages and limitations have been recently reviewed [13]. However, to date, tissue biopsy of OPMD remains the current standard for **diagnosis and histopathological grading** of OPMD to guide clinical management [14–15]. Unfortunately, it is challenging due to a high degree of inter- and intra-observer variability, with kappa values for such assessments ranging from 0.17 (poor agreement) to 0.78 (good agreement) [16], thus limiting the value of grading dysplasia as a predictive factor for malignant transformation of OPMD. Although several risk **biomarkers** on biopsy specimens have been proposed to improve patient stratification, they are not routinely used in patients due to lack of standardization and validation in large patient populations [17]. Finally, biomarkers in different types of specimens have been proposed to improve the identification of patients with OPMD at high risk of oral cancer [18], such as saliva [19] and buccal brushing, both of which have the advantage of being minimally invasive [20] but show poor accuracy and are not used in clinical scenarios [21]. Again, they are not routinely used due to insufficient evidence [22–23].

In medicine, artificial intelligence (AI) has recently emerged as a tool to assist clinicians in diagnosis, transforming current clinical workflows and providing new tools to improve diagnosis and treatment strategies. In general, these machine algorithms aim to learn patterns from large collections of data to mimic human behavior, in some cases revealing hidden correlations missed by human observation [24]. Today, Machine Learning (ML) algorithms have been successfully applied to a wide range of applications and data sources, including genomics, histology, radiology, telemedicine, electronic health records, robotics, and others [25, 26]. In the context of OPMD, ML has recently been proposed to improve the management of patients with OPMD. By analyzing and correlating different data sources, such as patient demographics, clinical history, imaging scans and histopathological findings, ML can identify high-risk patients [27]. Furthermore, ML models allow for the independent assessment of each patient by considering clinical history, biological/molecular profiles, and drug response. This could aid patient management by creating personalized treatment plans and predicting potential responses to treatment, ultimately improving patient survival rate and quality of life [28].

In this book chapter, we aim to briefly introduce how AI has the potential to transform clinical workflows for OPMD diagnosis and OSCC prevention. This chapter serves as a reference for both clinical and computational researchers, providing insights into current applications, challenges, recommendations, and potential of ML algorithms in the context of OPMD. We do not delve into complicated mathematical and complex concepts, instead we focus on a reader-friendly style for easy comprehension. The chapter is divided into three main sections. The first section is a description of what AI is through its history, the main branches (*e.g.* machine learning and deep learning), and how AI is applied to medicine in general. The second section is a state-of-the-art review of ML models in OPMD and OSCC prevention. The final section is a general overview of the current challenges of AI in medicine, how some of these challenges are being addressed, and general recommendations. For better conciseness, a list of abbreviations is given in Table 2.2.

**Table 2.2** List of abbreviations

| Term | Abbreviation |
|---|---|
| Oral Potential Malignant Disorder | OPMD |
| Oral Squamous Cell Carcinoma | OSCC |
| Artificial Intelligence | AI |
| Machine Learning | ML |
| Neuronal Networks | NN |
| Convolutional Neuronal Network | CNN |
| Deep Learning | DL |
| Recurrent Neuronal Network | RNN |
| Long Short-Term Memory Networks | LSTM |
| Natural Language Processing | NLP |
| Artificial Intelligence in Medicine | AIM |
| Support Vector Machine | SVM |
| Whole Slide Image | WSI |
| Explainable Artificial intelligence | XAI |

## Artificial Intelligence Definition Throughout Its History

Today, the term AI is used in almost every industrial and scientific field, making it difficult to identify any discipline that is not considering how to apply these algorithms to its operations. AI has significantly changed our understanding of the world, providing solutions that were unthinkable just a few years ago and often beyond human capabilities. To better understand AI, let's explore its history and evolution. A timeline of key AI events is shown in Fig. 2.1.

The concept of machines that mimic human intelligence began in 1942 with *the Enigma machine* created by Alan Turing. This machine was used during the Second World War to break the Enigma code used by the German military to communicate [29]. The way *the Enigma machine* broke the German codes, a task considered impossible by even the most brilliant mathematicians, inspired Turing to publish a seminal paper, "Computing Machinery and Intelligence", in 1950 [30]. In this paper, Turing expressed his idea of machines exhibiting intelligent behavior and proposed the *imitation game*, a simple test to determine a machine's ability to imitate human intelligence, a test that is still used today. In simple terms, the test involves interacting with a human and a computer simultaneously. If it is impossible to determine which responses come from the machine, then the machine is considered intelligent.

The term AI was first introduced in 1956 at the Dartmouth Summer Research Project on Artificial Intelligence. The conference brought together scientists from a variety of fields to investigate how machines could be made to simulate aspects of intelligence [31]. John McCarthy and Marvin Minsky, who hosted the meeting, formulated the AI conjecture: "Any aspect of learning or any other feature of intelligence can, in principle, be described so precisely that a machine can be made to simulate it." [32].

The Dartmouth conference served as a catalyst for researchers and governments to engage with the AI field over the following two decades, utilizing both human

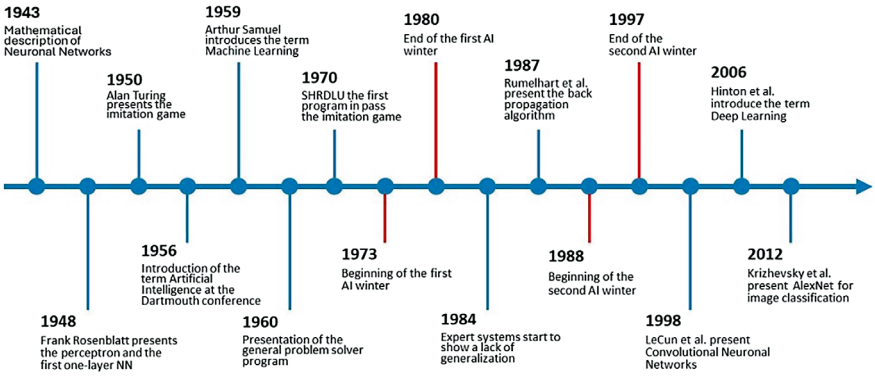


**Fig. 2.1** Timeline of AI evolution

and financial resources. Early AI examples that were successful included: 'The general problem solver program' [33] in 1960 was able to solve simple games such as the towers of Hanoi. In 1966, Eliza [34] was developed, which simulated a psychotherapist and was one of the first programs to pass the Turing test. In 1970, SHRDLU [35] was created, the first system to understand simple natural language. In 1959, Arthur Samuel included the term ML for the first time in the paper entitled "Some Studies in Machine Learning Using the Game of Checkers" [36]. In this paper, he described a program that learns and improves its performance through past events to play the checkers game. He later gave a more detailed definition of ML as the "field of study that gives computers the ability to learn without being explicitly programmed". However, by 1973, the difficulties of achieving human-level intelligence, coupled with the limitations in computational resources to develop more robust algorithms, led to a decline in funding resources and enthusiasm among researchers in the field of AI. These events marked the beginning of a period known as the "first AI winter", characterized by a significant slowdown in the advancement of the field.

This period concluded in the 1980s with the provision of renewed funding for AI research, initially by the Japanese government and subsequently by the U.S. Defense Advanced Research Projects Agency (DARPA). Following this, a significant advancement was made in AI algorithms with the advent of expert systems designed to solve specific problems utilizing Boolean logic (true/false). In 1984, the lack of generalizability when applying these models to problems from similar domains was identified as a significant challenge [37]. Furthermore, the rigid programming structure made it impossible to add the uncertainty inherent to complex problems. These limitations shifted research focus towards alternative approaches that better model human behavior, leading to the resurgence of a special kind of model based on artificial neurons. In fact, the concept of simulating biological Neuronal Networks (NN) originated in 1943 with McCulloch and Pitts [38], who mathematically described a simple unit composed of a soma and an axon connected to other neurons. After a specific activation threshold is reached, this unit can initiate an output signal (an example of this component is shown in Fig. 2.2).

In 1958, Frank Rosenblatt formalized this concept mathematically in 1958, introducing the term 'perceptron' for these basic processing units and presenting the first one-layer neural network, consisting of a parallel arrangement of neurons [39]. However, in 1969, limitations in perceptron were identified due to the Boolean logic used at that time, leading to a decline in research interest for the next two decades [40]. This decline in interest in NNs was reversed in 1986 with the publication of a seminal paper by Rumelhart, Hinton, and Williams. In this paper, the authors presented a different approach to optimizing NNs, based on minimizing the network errors. One year later, they extended their ideas, introducing the backpropagation algorithm, a method for iteratively updating each neuron value in order to optimize a specific target [41]. This algorithm is still regarded as a cornerstone of AI today. Despite the considerable expectations and enthusiasm surrounding NNs, the technology available in the 1980s was insufficient to scale these algorithms, resulting in a loss of their ability to learn more complex representations. This decline in interest

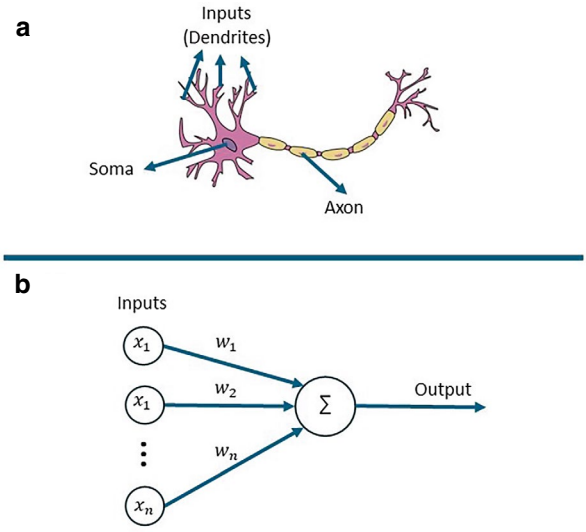


**Fig. 2.2** (**a**) Biological neuron presented by McCulloch and Pitts, (**b**) artificial neuron presented by Frank Rosenblatt

marked the beginning of the so-called "Second AI winter", which lasted for approximately a decade.

The success of IBM's Deep Blue chess-playing program in defeating the world chess champion Gary Kasparov is often regarded as a pivotal turning point in the field of AI marking the end of the second AI winter [42]. Although Dewinter [was an expert system, its triumph demonstrated the potential of computational processing power, with the ability to analyze 200 million possible moves per second. This hardware revolution enabled researchers to explore more complex NN models and utilize large datasets.

A seminal example was the work presented by LeCun and colleagues in 1998, which revolutionized the field of computer vision [43]. They presented a special kind of NN called convolutional neuronal networks (CNN), a matrix of neurons that allows the learning of several spatial patterns in one unique step. A key advantage of CNN compared to contemporary ML algorithms was their ability to automatically extract features from data, eliminating the need for manual feature engineering, a previously time-consuming human task.

Another significant development occurred in 2006 with the formal introduction of the term "Deep Learning" (DL) to describe NNs with multiple hidden layers [44]. However, arguably the most significant event in the field was the work presented in 2012 by Krizhevsky et al. This introduced AlexNet [45], a CNN trained on 1.2 million images across 1000 different classes, which outperformed previous implementations by reducing the classification error by 10.9%. The manner in which AlexNet was able to automatically learn complex patterns at different levels through its convolutional layers and the significant difference in performance, led to a shift in interest towards DL, which persists to this day.

In conclusion, the concept of AI has evolved considerably over time, with a multitude of definitions and subfields emerging as the field expanded. This makes AI one of the most challenging terms to define and comprehend. As illustrated in Fig. 2.3, AI is a broad concept that encompasses any aspect exhibiting human intelligence behavior, not limited to a specific set of algorithms. It encompasses ML and DL. However, AI does not necessarily involve a learning step from data. A case in point is the expert system Deep Blue, which does not necessarily learn from data but instead relies on complex rule-based systems. Similarly, self-driving cars are not necessarily learning machines, but rather rely on complex rule-based systems.

In contrast, ML algorithms are specifically designed to learn from data through training steps (Fig. 2.3). They map inputs (patterns or features) to produce specific outputs. These models often include a preprocessing step where the data is cleaned and formatted for the algorithms. Common ML algorithms include decision trees, logistic regression, principal component analysis, and k-nearest neighborhoods, among others.

DL is a more advanced branch of ML inspired by biological neural networks (Fig. 2.3). DL models can learn complex patterns through multiple layers without the need for explicit programming. Unlike some ML algorithms which may require a preprocess step, DL models can often learn complex patterns directly from raw data, eliminating the need for a separate preprocess step, as shown in Fig. 2.4. This flexibility has led to the development of a range of specialized DL architectures for specific tasks. These include convolutional neural networks (CNNs) for images [46], recurrent neural networks (RNNs) for managing sequential data in the form of text, speech or time series data, and long short-term memory (LSTM) networks, which enable the learning of long-term dependencies for large video, audio, or text sequences [47].

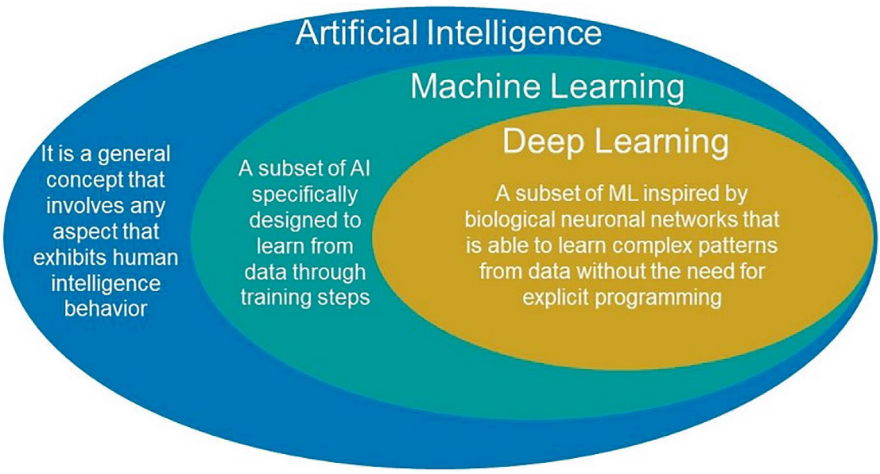


**Fig. 2.3** Artificial intelligence and its subfields. Machine learning is a subfield of artificial intelligence, and deep learning is a subfield of machine learning and, in turn, a subfield of artificial intelligence

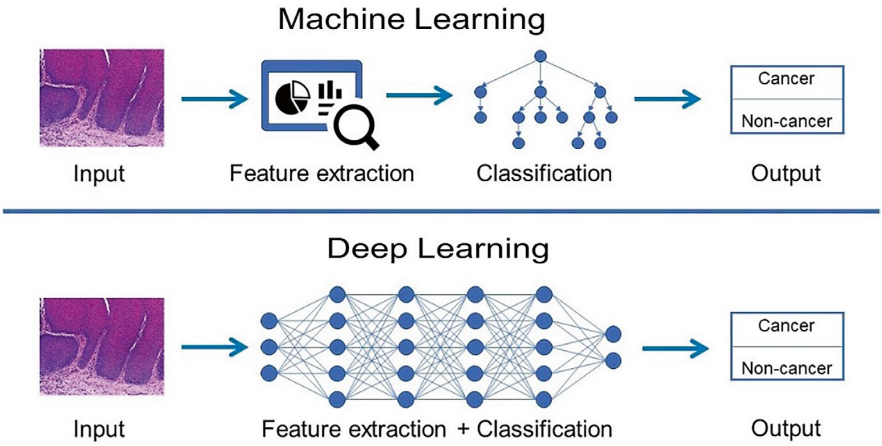


**Fig. 2.4** It is often the case that machine learning algorithms require a preprocessing step prior to the training step. In contrast, deep learning algorithms are capable of automatically extracting features from raw data, thereby eliminating the need for the preprocessing step. Such algorithms are designated as end-to-end

## AI in Medicine

Over the past two decades, the integration of AI into medicine has led to significant advancements in the diagnosis, surgical procedures, and development of pharmaceuticals. The advent of ML and DL has enabled a more comprehensive understanding and modelling of the complexities of several diseases, rather than the previous approach of addressing specific conditions through expert systems. The digital revolution has also played a pivotal role in this progress. The digitization of medical records, diagnostic tools, and patient data has enabled the enrichment and fine-tuning of AI algorithms.

Limited progress in applying AI to medicine was achieved during the early years, primarily due to limited data availability and the lack of standardization of clinical protocols, making it difficult to explore AI solutions. It was not until 1975 when Rutgers University started to introduce AI in Medicine (AIM) workshops to share ideas, models, and prospects for the field between AI specialists and clinicians [48]. Three years later, the first prototype was presented that demonstrated a certain viability in the application of AI in medicine, a glaucoma consultation program [49]. Another representative work was Dxplain in 1986 after the second AI winter, a decision support system to give a list of possible differential diagnoses based on the symptoms [50]. In the 1990s, several efforts were made to standardize and collect medical data. Examples of such initiatives include the Unified Medical Language System (UMLS) [51], which standardized medical terminologies and vocabularies, and Medline with its search engine PubMed, a vast repository of biomedical literature [52], among others.

The 2000s witnessed a revolution in AIM driven by the emergence of DL models. Advances in computational power and the generation of massive volumes of data allowed scientists to overcome several limitations of the early approaches. The ability of DL models to analyze and extract complex disease patterns from different sources opened a new era of healthcare. Clinicians now have powerful AI tools that can not only improve patient outcomes but also transform how some interactions are performed. For example, AI-powered sensors can continuously monitor patients with cardiac diseases or diabetes, notifying clinicians of any alterations in their condition [53]. Similarly, smartwatches equipped with AI can warn users of potential changes in heart rhythm or body temperature [54]. Furthermore, telemedicine powered by AI tools is expanding healthcare services to remote areas [55].

AI has been explored in several clinical areas for outcome prediction and as a diagnostic tool. For instance, in dermatology, AI systems have achieved accuracy comparable to dermatologists in classifying skin cancer images [56]. Similarly, in gastroenterology, AI is improving the diagnosis of polyp lesions [57]. Furthermore, in ophthalmology, AI serves as an autonomous screening tool for retinopathy, helping to prevent blindness [58]. In neurology, AI can assist in the early diagnosis of Parkinson's disease by analyzing handwriting or gait patterns [59]. In histology, AI can reduce sample preparation by generating virtual stains from unstained tissue sections [60], among other applications. Furthermore, advances in natural language processing (NLP) enable AI to exploit sequential text information. This facilitates tasks such as automatic identification of risk events in clinical incident reports and providing feedback to clinicians based on patient comments. NLP also powers chatbots capable of handling administrative tasks and providing patient support [61].

## AI in Oral Potential Malignant Disorders (OPMDs)

The field of oncology has been extensively investigated by AIM in recent years. The necessity to enhance our comprehension of the biological intra- and inter-patient heterogeneity, to diagnose the disease at an earlier stage, to improve patient stratification, to personalize multimodal therapy, to enhance patient follow-up and to treat recurrent disease when it occurs, has naturally led to the application of these techniques in OSCC and OPMD, as they have been applied in other anatomical sites [62].

This section will present a summary of recent reviews [63, 64] that have been conducted on the applications of AI in the field of oral cancer and OPMD. A summary of these works can be found in Table 2.3.

Both papers discuss supervised and unsupervised learning, two main approaches to learning patterns from data. Supervised learning algorithms are capable of learning strong correlations between data (inputs) and predictions (outputs) but require labelled data for training. This labelling can be time-consuming and resource-intensive, especially in domains such as medicine. In contrast, unsupervised learning can automatically identify hidden structures from the data without the need for

**Table 2.3** Summary of results presented in the scoping and systematic review

| Authors | Search criteria | Total works studied | Categories | Method |
|---|---|---|---|---|
| García-Pola M, et al. | Improve early diagnosis of OPMD/oral cancer | 36 | Mobile phone<br>Medical imaging<br>Auto-fluorescence imaging<br>Exfoliative cytology<br>Predictor variables | Focused review (PRISMA recommendations, CRD42020 218675) |
| Mahmood, H, et al. | Studies that utilized whole slide images of human tissue sections | 11 | Detection of OPMD<br>Detection of OSCC<br>Detection of oropharyngeal squamous cell carcinoma | Focused review (PRISMA recommendations, CRD42019 153,023) |

labels. Nevertheless, these models are less effective in certain tasks that require specific classifications or predictions of future outcomes.

The first work, a scoping review, was conducted to answer the question: "What are the applications and performance of artificial intelligence in the early diagnosis of oral cancer?". Articles published between 2000 and 2020 were retrieved from PubMed, Web of Science, and Google Scholar using search terms such as "oral cancer", "oral precancer", "oral potentially malignant disorder", "artificial intelligence", "deep learning", "machine learning", among others. A total of 1551 articles were identified in the initial search. Following the application of inclusion and exclusion criteria, only 36 articles were included in the final analysis. Articles related to radiology, magnetic resonance, metastasis, biomarkers, planning of treatment and survival were removed.

The authors classified the results into five distinct categories of studies:

**Mobile Phone Technologies** In this group, six works were identified that share the same idea: to improve the diagnosis of OPMD by connecting dental professionals with non-expert field health workers through mobile phones supported by AI. All of them use DL for classification and detection tasks, with specificities ranging from 44% to 92%. An important point is how AI can improve clinical workflows, not only by providing support, but also by connecting specialists.

**Medical Imaging Techniques** The nine studies that were investigated utilized a variety of medical imaging techniques, including the classification of normal and abnormal mucosa, the identification of solar versus non-solar cheilosis, the differentiation between different types of OPMDs, such as oral lichen planus, leukoplakia and normal tissue, and the distinction between normal tissue and OSCC, among others. In this case, the two predominant algorithms were the support vector machine (SVM) and DL, which demonstrated an average accuracy of 95%.

***In Vivo* Optical Imaging Using Auto-Fluorescence Imaging** A total of eleven works were presented in this case, including two that were implemented using

mobile technologies. The objective was to include a non-invasive luminance to reveal hidden patterns using AI algorithms for the diagnosis of OPMD and/or OSCC. Algorithms such as CNN, SVM and principal component analysis for dimension reduction were used for classification.

**Exfoliative Cytology** Nine articles were published on this topic, with a focus on liquid-based cytology, scrape biopsies, and brush biopsies for the screening and diagnosis of oral lesions. Algorithms such as SVM, K-nearest neighbors, random forest, and CNN were employed to distinguish OPMD from OSCC or to classify different types of OPMDs. An intriguing contribution by Sumsum et al. presented a mobile microscope for telecytology. The system is capable of automatically scanning cytology slides, uploading images to a web server, and performing cytological assessment using a DL model [65].

**Predictor Variables of Datasets** The final group comprises five studies. The primary objective is to identify and combine the most representative features from disparate data sources, including demographic characteristics, clinical features, and histopathological features, in order to construct a predictive model for the early detection of oral cancer.

The second work, a systematic review, focused specifically on articles evaluating the application and diagnostic accuracy of AI methods for histopathological assessment and grading of OPMD and head and neck cancer. The review only considered studies that utilized whole slide images (WSI) of human tissue sections. An initial search identified 315 articles, but only 11 met the inclusion criteria. The search yielded 315 articles from Medline, Scopus, and Web of Science between 2009 and 2020. Articles were excluded if they did not utilize WSI, employed modalities other than histopathology, or predicted prognosis or treatment efficacy.

The authors classified the reviewed studies into three groups based on the targeted outcome: the detection of OPMD, OSCC, or oropharyngeal squamous cell carcinoma. The **first group** comprises six studies. One study employed a random forest algorithm to quantify changes in cell nuclei appearance. The remaining five studies aimed to differentiate between normal tissue, oral submucous fibrosis with dysplasia or atrophy. The algorithms implemented in this group varied, including DL, k-nearest neighborhoods, random forest, and SVM. The **second group** consists of three studies: one for segmenting epithelial, subepithelial, and keratin layers, another for classifying cancerous cells, and the last one for segmenting and classifying OSCC using an antibody targeting CD34, which has been associated with a critical prognosis factor in oral cancer. The AI algorithms in this group included random forest, DL, SVM, and clustering algorithms (which group similar data points). The **final group** included one study for classifying epithelial and stromal tissue using clustering algorithms. An additional study, not assigned to a specific group, used SVM for fibroblast segmentation.

Limitations of data quality and proper validation are common to both reviews. For example, data sets are collected from a single center and lose diversity and

generalizability when models are tested using data from different sources. Another data issue is the lack of careful annotation without input from multiple pathologists to reduce subjective and possible human bias. The weakness in validation is also shown in some papers when the data used to optimize the model is the same to test the result, showing a high precision that does not reflect a real impact. Furthermore, some results do not show overall precision and cannot be compared with other approaches, thus losing reproducibility.

In summary, the advancement of ML and DL has enabled the application of these algorithms to a diverse range of clinical and histopathological challenges related to OSCC and OPMD. For instance, in automated image analysis, AI algorithms can identify lesions through various sources, including smartphones and imaging devices, providing new rapid screening alternatives that facilitate early detection of the disease. As a support system, AI may assist clinicians in the diagnosis and management of OPMD, providing real-time recommendations based on current clinical workflows. Furthermore, these systems can analyze individual patient information in different modalities (history, images, risk factors) to provide personalized treatments and plans. AI may also provide non-invasive ways to inform oral cancer risk, thus facilitating follow-up and timely interventions. In general, AI is a valuable ally in the fight against oral cancer and related disorders. As research and development in this area continues to advance, the potential for AI to revolutionize diagnosis, treatment and patient care in oral oncology remains promising.

Following the publication of these two reviews, we summarize here some important work in the field of OPMD/OSCC. For example, Tanriver et al. [66] and Wartin et al. [67] proposed DL-based algorithms for real-time detection and classification of OPMD using photographic images. These models provide a low-cost and non-invasive alternative to assist in the screening process, achieving F1 scores of 0.86 and 0.95, respectively. Birur et al. [68] proposed a dual-mode imaging technique for the early detection of OPMD using autofluorescence and white light images acquired from a large patient cohort of 5025 individuals. Their study involved a modified smartphone capable of capturing both imaging modalities and a mobile app that classified the images using a cloud-based DL approach, demonstrating a sensitivity of 0.87. Adeoye et al. [69] implemented a DL algorithm to predict the risk of malignant transformation of OPMD using medical records of patients with at least 18 months of follow-up. The authors provide a public website where users can enter 26 variables to obtain metrics for OPMD malignant transformation risk.

## Current Challenges of AI in Medicine

Despite the remarkable progress of AIM, several challenges remain that require collaboration between scientists, clinicians, and policy makers. Regardless of the application, issues such as data quality, implementation regulations and ethical concerns are common to all medical fields.

## *Data Quality and Bias*

Current AI models such as DL rely on large amounts of data to identify complex patterns. This data can be labelled (with specific classifications) or unlabeled (uncategorized). In the case of labelled data, it requires high quality in terms of both the data itself (*e.g.* high-resolution images) and the annotations or labels (*e.g.* accurate and consistent labelling). However, the collection of such information in medicine can require expensive equipment and human resources. As a result, alternative solutions have emerged to avoid the need for human labelling. One example is unsupervised learning, where models discover patterns or groups in data without explicit human intervention. Another is weakly supervised learning, which allows models to learn from smaller datasets or data with noisy or less reliable labels [70].

Another problem arises when models are trained using data from a single source, such as a single institution. This can lead to bias, where AI models perform well under certain conditions but show unexpected behavior when data comes from different sources. Indeed, generalization is an important challenge in AI applications and a critical factor in the diffusion of applications. There are several solutions to address this bias. First, using data from different collection sources enriches the model with different experiences (*e.g.,* images from different clinical centers). Second, incorporating labels with consensus from multiple experts reduces the potential bias introduced by a single annotator. In addition, the use of regularized terms helps models avoid overfitting to specific biases present in the data by penalizing overly complex models and encouraging them to learn more generalizable patterns [71]. Other data challenges include differences between retrospective and prospective approaches when dealing with real-world, large data sets (*e.g.* histopathological images) that require expensive and complex computational resources, selection of best metrics, and others.

## *Human Barriers and AI Adoption in Healthcare*

While AIM can deliver exceptional results, it is critical to ensure that these algorithms reach and benefit patients. Increasing user confidence while reducing human barriers is important to bridge the gap between technology and clinical applications. Even when algorithms perform exceptionally well, human resistance can hinder their adoption. AI systems need to be transparent, easy to use and easy to integrate into clinical workflows. Therefore, scientists need to include user-friendly instructions and interfaces in their implementations through continuous updates based on feedback from clinicians [72].

Transparency is another important factor. While many AIM research papers have been published, few have demonstrated their performance in real-world clinical settings. Clear and transparent reporting of how these algorithms are implemented and how they improve clinical workflows is essential to build user confidence and drive adoption [73].

Another key point, and perhaps the most difficult to achieve, is accountability. Understanding the reasoning behind the AI's conclusions provides a deeper insight into the disease and promotes confidence in the decision. However, current algorithms such as DL are often considered 'black boxes' because it is difficult to understand how they reach their conclusions. The emerging field of Explainable AI (XAI) aims to address this challenge by providing insight into the decision-making processes of models. This allows us to identify and correct potential biases. Explainable AI helps build trust in AI systems by ensuring fairness and improving overall performance through better debugging [74].

### *Ethical and Legal Considerations*

The rapid development of AIM is outpacing current legal and regulatory frameworks. Integrating AIM into clinical workflows raises concerns about unforeseen consequences, such as who is responsible for AI-driven misdiagnoses. Public regulatory frameworks are needed to standardize AIM applications without putting patients at risk. One example is the recent guidance from the US Food and Drug Administration (FDA) to ensure that safe and effective AI devices can be efficiently delivered to patients [75]. Another example is the recent EU AI Act, the first regulatory framework for AI that sets requirements and obligations for developers based on the risk level of the AI application [76].

Patient privacy is also critical when dealing with sensitive clinical data. One promising solution is to move from centralized data storage to distributed data centers. Instead of storing data in a single location, distributed data center storage allows a small portion of the data to be stored in different locations, reducing the risk of cyber-attacks and information filtering. Federated learning provides an additional layer of security [77]. This technique allows hospitals to collaboratively train AI models without ever sharing the raw patient data itself. The AI models learn patterns in each hospital from its local data and only share the 'learned patterns', enabling collaboration while ensuring patient privacy.

## Future Directions

Advances in hardware technology, coupled with digital transformation, are creating new ways to screen, diagnose, assess, and manage OPMD and OSCC. For example, combining data from multiple sources, such as medical records, histopathological information, and medical images, can provide a more comprehensive understanding of risk assessment and facilitate personalized patient assessments. To fully realize this potential, collaboration between hospitals and data scientists is essential. Standardizing clinical procedures and ensuring transparent and easy sharing of patient information will be essential to maximizing the benefits of AI-powered digital pathology.

The emergence of new technologies is also creating new ways to analyze and understand the interactions between OPMD and OSCC transformation. For example, genomic analysis can provide a more complete picture of the disease to inform treatment decisions. In addition, advances in digital tools such as infrared imaging can go deeper than traditional visual analysis, providing cellular chemical composition and interactions to support the development of new personalized therapies [78]. In this context, AI can act as a bridge, linking current analysis of morphological features with biological or genomic information. This will allow AI to present pathologists with only the most relevant and insightful information, allowing them to make more informed diagnoses.

## Conclusions

AIM shows great promise as an assistive tool to improve the diagnosis, detection, and management of OPMDs and OSCC. The high flexibility of current AI models allows for integration into various workflows, including diagnosis, administrative tasks, and even patient follow-up. However, realizing this potential requires a collaborative effort between clinicians, scientists, and policy makers to overcome data collection challenges, human resistance, and ethical considerations. In addition, successful implementation of AIM requires not only a deep understanding of OPMD, but also the needs of clinicians. This can be achieved through continuous refinement based on user feedback. Future directions include the exploration of more complex diagnosis by incorporating different data sources into the training process and the integration of new technologies to better understand biochemical interactions.

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# Chapter 3
# Treatment of OPMD and First Results of Immune Checkpoint Inhibitors

**Paolo Bossi and Erika Stucchi**

## Introduction

Oral potentially malignant diseases (OPMDs) are a group of oral mucosal lesions with an increased risk of malignant transformation. OPMDs include many types of lesions (Fig. 3.1), like erythroplakia, leukoplakia, oral lichen planus, and proliferative verrucous leukoplakia, characterized by different clinical presentations, etiology, and histology. The main risk factors for OPMDs are linked to those of oral squamous cell carcinoma (OSCC) and are tobacco, alcohol, derivatives of betel nuts, chronic mucosal inflammation, and oral mucosal trauma from teeth and prosthetic devices [1].

Genetic mutations such as P53, minichromosome maintenance (MCM) complex proteins, and cytogenetic and epigenetic alterations contribute to their progression in oral cancer [2]. To date, despite the equality of risk factors, there are no clinical or histological factors that could predict the risk of OPMDs' malignant transformation to oral squamous cell carcinoma [3], except for previous OSCC and WHO classifications [4]. In these types of lesions, it appears essential to plan active surveillance, particularly for patients with risk factors, and to do early diagnosis with a visual exploration of the oral cavity and tissue biopsy [5]. In fact, studies have demonstrated that a diagnostic delay directly influences the baseline tumor classification (TNM) and patient's survival from OSCC [6]. To facilitate early diagnosis, especially when the objective examination raises doubts, adjuvant techniques have been studied such as oral cytology, salivary biomarkers, and light-based techniques

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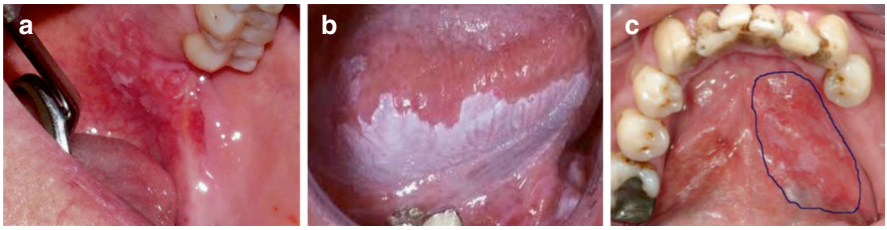


**Fig. 3.1** Examples of oral potentially malignant diseases. (**a**) erythroplakia; (**b**) leukoplakia; (**c**) oral lichen planus

(auto-fluorescence, chemiluminescence, and spectroscopy) [1]. Cytology is the additional test with the higher diagnostic value, but the cost and possible delay of final tissue diagnosis by biopsy must be considered. On the other hand, spectroscopy showed the highest sensitivity and accuracy, with limitations in some anatomical sites. However, the evidence on the role of these adjunctive tests demonstrated that none of the tests could replace tissue biopsy [7, 8].

However, after diagnosing the lesions, we must establish a proper treatment plan. Surgical resection is the standard treatment, aiming to remove all the affected epithelium of the oral precancerous lesion. It is recommended, during the surgery, to leave a free margin of 1–2 mm. The most commonly used techniques are the cold-blade scalpel or electrocautery excision [9]. Recently, clinicians have also reported benefits in bleeding, swelling, and pain management from the use of CO2, Nd:YAG, Er:YAG, or diode lasers, as well as photodynamic therapy. It appears to be a good treatment option for oral lichen planus and oral fungal infections [1]. Despite complete surgical removal of the lesion, there is a high probability of recurrence and the development of malignant transformation. This could be explained by the theory of "field of cancerization," an area constantly exposed to risk factors that develops molecular alterations and consequently tissue dysplasia increasing the risk of second malignancies. So, for a patient with an oral precancerous lesion, close and long-term surveillance strategies are mandatory.

## Chemoprevention

Because of a high rate of recurrence and second primary tumors, researchers came up with an idea of chemoprevention. This means using a systemic agent to stop the process of carcinogenesis along with surveillance [10]. Based on this concept, researchers have conducted several studies, but the results have been disappointing. One of the first agents studied was retinoic acid. Hong et al. randomized patients with leukoplakia to receive 13-cis-retinoic 1–2 mg/kg/day or placebo for 3 months. Sixty-seven percent of those given the study drug (16 patients) had a clinical response, compared to 10% (2 patients) of those given the placebo. Furthermore, in the group that received the study drug dysplasia was reversed in 54% of the patients, while in the group that received

placebo that occurred in 10% [11]. Despite these positive results, the trial also showed some negative issues, such as toxicity, which was not negligible for a population of patients with non-malignant lesions, and the short-term clinical benefit. In fact, more than half of patients relapsed within only 3 months after treatment ended. Other researchers also tested the effects of beta-carotene and vitamin C in a non-smoking patient population with leukoplakia. The overall clinical response rate in the experimental arm was 17.4% (4/23) and 4.3% (1/23) in the placebo arm. During the median 60-month follow-up period, two subjects in the experimental arm and three in the control arm developed oral cancer, showing no significant differences between the two groups [12]. In order to minimize systemic toxicities, the cyclooxygenase inhibitor ketorolac was studied as an oral rinse in oropharyngeal leukoplakia in a randomized, double-blind, placebo-controlled trial. The study demonstrated no significant difference in the change of histology between the use of ketorolac rinse and the placebo [13]. Lastly, in this setting, the efficacy of erlotinib was studied. The "EPOC trial" evaluated the activity of erlotinib in patients affected by high-risk OPMDs (defined as loss of heterozygosity [LOH] on chromosome 3p14 and/or 9p21 with a history of oral cancer or LOH on chromosome 3p14 and/or 9p21 and additional LOH on the short arm of chromosome 17, 8, and 11, and on chromosome 4q, or 13q). Erlotinib didn't improve cancer-free survival in high-risk patients with LOH-positive OPMDs. However, the study showed a correlation between increased EGFR gene copy number (target of the intervention), LOH-positive status and lower oral cancer free survival. Despite this negative result, this study is a landmark study because it validated LOH as a marker of oral cancer risk, demonstrating an association with increased EGFR copy number [14]. The 2-hit (LOH and EGFR/chromosome 7 polysomy) genomic instability-driven model may explain the lack of erlotinib efficacy found in the EPOC trial. These high-risk OPMDs may have already accumulated additional changes, leading to the persistent activation of signalling pathways operating downstream or independently of EGFR.

## Immunotherapy

After these unsuccessful results, a deeper analysis was attempted, in particular investigating the peri- and intratumoral immune activity. Research has demonstrated that changes in the number of tumor-infiltrating CD8+ cells can impact the malignant transformation of OPMDs. An increased CD8+ cell infiltrate was noted in premalignant lesions that did not evolve into cancer versus those that preceded malignant transformation [15]. On the other hand, it has also been demonstrated that PD-L1 is highly expressed in premalignant lesions progressing to cancer [16, 17]. The OPMDs with lower infiltration of CD3+ T-cells and high Th1 cell levels seem to have a higher risk of malignant transformation [18]. In patients with OPMDs, systemic inflammatory activity was also detected, with increased levels of TNF-alpha and pro-inflammatory cytokines both in plasma and saliva [19]. Therefore, the OPMDs could be considered the equilibrium phase of the immunoediting concept, a dynamic process between tumor cell growth and the

immunosurveillance of the immune system. An imbalance in the immunosuppressive microenvironment could be the possible key to malignant transformation, suggesting a potential benefit of using immunotherapy as a preventive drug. The first study to investigate the effectiveness of the immune checkpoint inhibitors in this setting was the phase II clinical trial conducted at Dana-Farber Cancer Institute in Boston by Glenn J. Hanna et al. [20]. The study included 33 people who had high-risk proliferative verrucous leukoplakia, which was defined as having at least two multifocal lesions, two or more contiguous lesions measuring more than 3 cm, one lesion measuring more than 4 cm with any level of dysplasia, or at least one localized leukoplakia with moderate dysplasia. Patients underwent a pretreatment biopsy and then received 4 doses of nivolumab (480 mg intravenously) every 28 days, followed by a re-biopsy and intraoral photographs at each visit (Fig. 3.2). The primary endpoint was the response, evaluated with a modified composite scoring system determined by the sum of the percent change in composite (both clinical and pathologic) of the target lesion. Major response was a decrease of more than 80%; partial response was a decrease of 40% to 80%; and progression of disease was defined as an increase of 10% or more in the composite score or an OSCC diagnosis.

Twelve patients (36%) had a response by composite score regardless of PD-L1 status (three of them with major responses), and four had progressive disease. Nine patients (27%) developed OSCC during the trial. The 2-year cancer-free survival rate was 73%. The safety results were in line with those obtained in other studies with immune checkpoint inhibitors for head and neck cancer. Two patients (6%) discontinued because of toxic effects. Seven patients (21%) experienced grade 3 to 4 immune-related adverse events, including immune-mediated hepatitis and colitis requiring immunosuppression. Fatigue and diarrhea were the most common reported adverse events. These findings need to be carefully weighed against the potential for clinical activity, assessing the therapeutic risk-benefit ratio in precancerous lesions.

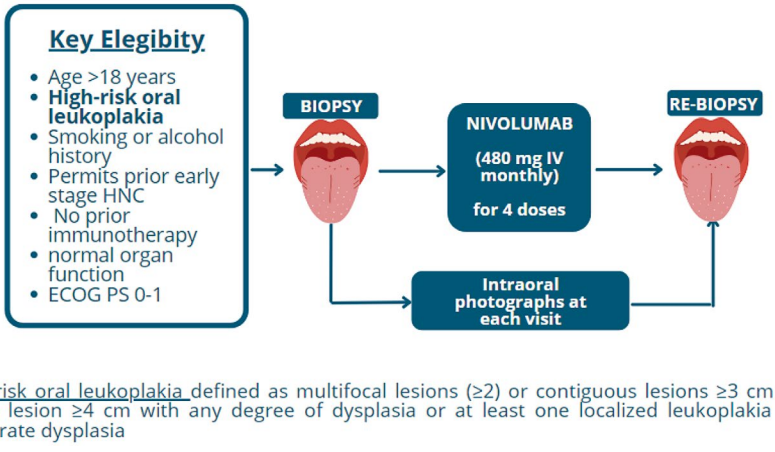


**Fig. 3.2** Study design of "Nivolumab for Patients With High-Risk Oral Leukoplakia: A Non-randomized Controlled Trial"

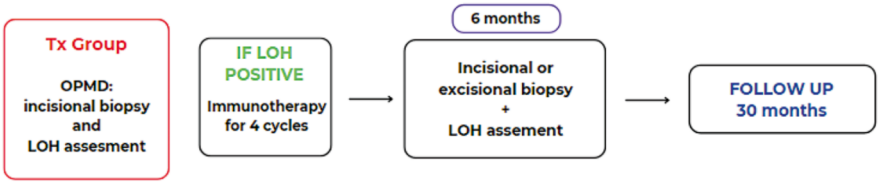


**Fig. 3.3** IMPEDE TRIAL study design. From Gurizzan C, Lorini L, Paderno A, Tomasoni M, Zigliani G, Bozzola A, et al. Immunotherapy for the prevention of high-risk oral disorders malignant transformation: the IMPEDE trial. BMC Cancer. 2021 May; 21 (1): 561. Licensed under CC-BY 4.0. http://creativecommons.org/licenses/by/4.0/

Currently, another ongoing study, called "IMPEDE" (NCT04504552), aims to analyse the role of immune checkpoint inhibitors against the malignant transformation of OPMDs. This is a phase II, open-label, single-arm trial involving Italian centers to treat oral premalignant lesions with any grade of dysplasia and being positive for loss of heterozygosity (LOH) [21]. The researchers select the patients with LOH to define a high-risk population for progression to oral cancer [22, 23]. The patients who test positive for LOH after an incisional biopsy undergo a short course of immunotherapy with 4 administrations of avelumab (800 mg every 2 weeks). After 6 months since treatment started, resection of the OPMD is performed, and LOH assessment will be repeated (Fig. 3.3 [21]).

The coprimary objectives of the trial are change in LOH status (positive to negative) of OPMD after 6 months since the start of immunotherapy and the recurrence or malignancy-free survival, for which the events of interest are the recurrence of OPMD with LOH or malignant transformation.

The safety of an immunotherapeutic approach, the change of histological grade of OPMD and the discovery of other genomic factors predictive to response to avelumab will be evaluated as secondary objectives. The data from the interim safety analysis are currently available. Of the 49 patients screened, 16 (33%) had LOH, and of these, 12 received immunotherapy (4 did not want to proceed with the pharmacological phase of the study). After a median follow-up of 14 months, 39 adverse events were reported, but of these, only 18 (in 6 patients) were considered immune-related. The most frequent side effect was oral pain. Only three grade 2 adverse events were described, namely amylase/lipase increase, psoriasis, and fever, and no grade 3–4 adverse events were reported. No patient stopped immunotherapy or delayed local excision for toxicities [24]. However, we must wait until the study is completed to have solid data on this setting.

## Conclusions

Although these early results on the use of immune checkpoint inhibitors to reverse malignant transformation of OPMDs are promising, there are many open questions still to be clarified. To date, the studies performed are phase II trials conducted with

a small number of patients and using clinical response or LOH reversion as a surrogate endpoint. To ascertain the benefit of immunotherapy in reducing malignant transformation, randomized trials will have to be designed. It is also fundamental to have long-term outcomes. At all times we should keep in mind that we are dealing with patients with premalignant lesions. The therapies used should have a low toxicity profile and should not alter or worsen the patient's quality of life. It is therefore essential to perform a proper selection of patients at higher risk of malignant transformation, either by clinical, histological, or molecular features, such as the LOH. A risk-stratification method could improve quality-adjusted survival outcomes for patients with OPMDs [25]. Ideally, the application of the concept of "precision medicine"to OPMD would imply changes in follow-up frequency or early intervention as compared with the current standard of care, based on the characteristics of the lesion and the individual patient. Possible future scenarios, in addition to deepening the efficacy of immunotherapy in this setting, will focus on new, more specific, effective, and less toxic treatment options, like intralesional injections.

The results of preclinical research need to be applied in the design of new trials, so that more tailored and focused strategies may be applied to prevent the risk of malignant transformation.

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# Chapter 4
# Innovations in Cell-Based Assays for Drug Discovery and Evaluation


**Ingeborg Tinhofer**

## Introduction

Head and neck squamous cell carcinoma (HNSCC) poses a considerable therapeutic challenge, especially in recurrent or metastatic (R/M) cases where treatment outcomes have seen limited progress. Historically, systemic therapies relied on platinum-based regimens, which remain the standard despite recent advancements in molecular targeted therapy. The addition of agents like cetuximab and immune checkpoint inhibitors has improved outcomes, but these options are still limited compared to other cancers where molecular analysis guides personalized treatment decisions. HNSCC lacks prevalent genetic drivers for tailored therapies, and reliable biomarkers for treatment selection are scarce, necessitating innovative strategies to enhance treatment efficacy. The high need for new approaches in drug development for HNSCC is further underscored by a recent review on the trends in FDA approvals of oncology therapeutic products [1], which identified HNSCC as one of the cancer types with the lowest number of new drug approvals over the last 25 years.

While traditional two-dimensional (2D) cell culture systems have been widely used in drug development due to simplicity and cost-effectiveness, their translational success is limited by their inability to replicate *in vivo* tumor characteristics. Consequently, efforts have shifted towards more sophisticated 3D culture systems that better mimic the tumor microenvironment. Personalized tumor models, including organoids, spheroids, and tumor tissue explants derived from patients, offer

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promise in tailoring cancer treatment approaches. Further exploration of these advanced models holds promise for enhancing our understanding of the pathobiology in HNSCC. Integrating advanced cell-based assays early in drug and biomarker development could expedite treatment optimization, potentially also benefiting HNSCC patients.

## Search Strategy and Selection Criteria

For this review focusing on recent advancements in cell-based assays for drug discovery and evaluation, a comprehensive literature search was conducted using PubMed. The search strategy included terms related to head and neck cancer, specifically "head and neck cancer", "squamous cell carcinoma of the head and neck", "head and neck squamous cell carcinoma", "oral cancer", "oropharynx cancer", "hypopharynx cancer" and "larynx cancer". Additionally, terms related to cell-based assays were included, such as "organoids", "spheroids", "organotypic", "patient-derived organoids", "explants" and "primary culture".

Articles published between January 1, 2019, and March 1, 2024 were considered. Original articles were prioritized using a search filter. Review articles were also cited to offer readers additional insights and resources beyond the scope of this review.

## Tumor Spheroids

In the 1970s, a shift from traditional 2D cell cultures to 3D environments for culturing cancer cells began, driven by the recognition of tumor heterogeneity once tumors reach detectable sizes. This heterogeneity gives rise to diverse cell subpopulations within the tumor, influenced by factors like inefficient vascularization, leading to the formation of microecological niches. These niches exhibit significant gradients of essential metabolites, oxygen and other factors, fostering the emergence of various cell phenotypes with altered responses to treatments due to selective pressures and genetic instability.

Modelling these abnormal tumor environments in *ex vivo* culture system presents opportunities for improved development of diagnostics and therapeutic approaches. Tumor spheroids, initially described by Sutherland et al. in the 1970s [2, 3], depict the phenomenon of cancer cells organizing into spherical multicellular structures under conditions that promote 3D growth in culture. The spheroid model promotes the expression of stem cell markers, such as Oct-4 and Nanog, which influences cell proliferation, survival, and migration. These models closely mimic the complexity of solid tumor microenvironments [4], including hypoxia, extracellular matrix interactions, pH fluctuations, nutrient availability, and drug penetration. The pioneering work by Sutherland and coworkers [2, 3] laid the groundwork for subsequent research exploring tumor biology using 3D culture models.

Over the past five years, tumor spheroids have been broadly used for drug development in HNSCC across various contexts. Table 4.1 provides an overview of these studies, including their objectives and key findings. Siemer and colleagues utilized spheroid cultures in conjunction with CRISPR/Cas9 knockout techniques to identify crucial molecular players involved in cisplatin resistance [5]. Through functional screens, they pinpointed leucine-rich repeat-containing protein 8A (LRRC8A), an ion channel protein implicated in drug efflux, as a potential key factor. Subsequent experimental treatments conducted in spheroids demonstrated that LRRC8A-mediated resistance could be overcome by administering nanoparticle-formulated cisplatin, suggesting a novel promising therapeutic strategy for tumors characterized by elevated LRRC8A expression [5]. Another study on chemoresistance showed that patient-derived spheroids provide a robust and reliable *in vitro* model to identify tumors harbouring a multidrug resistance phenotype [6]. Two further studies used spheroids as screening platform of potential radiosensitizing drugs. Independently, these studies unveiled that poly(ADP-ribose)polymerase (PARP) inhibition can enhance sensitivity to radiation, encompassing both photons and protons [7, 8], with a particularly pronounced effect observed in HPV-negative HNSCC tumor cells. In the study of Lee et al., it was also shown that screens for radio sensitizing drugs can be scaled up to high-throughput by using a 384-pillar/well platform [7]. After adoption for usage in combination with proton radiation, it was shown that testing spheroid responses to graded doses of protons in combination with drug screens is feasible [7]. Spheroids were also leveraged for the development of targeted drugs, with recent studies focusing on molecular drugs targeting H-Ras [9] and Notch signalling [10, 11]. Notably, in the study of Gilardi et al. the farnesyltransferase inhibitor tipifarnib specifically inhibited growth of *HRAS*-mutant but not *HRAS*-wt HNSCC cells in spheroid cultures, whereas its activity was diminished in monolayer culture settings, emphasizing the importance of utilizing appropriate assay formats to accurately interpret tumor dependence on the targeted signalling pathway [9]. The specific enrichment of tumor cells with a stemness phenotype in spheroid cultures has also been exploited to pinpoint potential druggable targets in subpopulation of tumor stem cells [12] linked with treatment resistance and a more aggressive disease course. This led to the identification of small-molecule Notch modulators as a promising therapeutic strategy in patients with intact Notch signalling, while tumors with low NOTCH activity showed higher sensitivity to JAK-selective drugs like ruxolitinib or tofacitinib [11].

The fact that patient-derived spheroids are clusters of broad-ranging cells including tumor-infiltrating immune cells makes them an ideal model for analysing the crosstalk between cancer cells and cells from the immune stroma. Recent studies have utilized spheroids to explore novel therapeutic strategies aimed at counteracting the immunosuppressive effects exerted by tumor-associated macrophages [13–15]. Mechanistic studies performed in a spheroid invasion model by Wu et al. revealed that tumor necrosis factor-alpha, secreted by M1 macrophages at the invasion front, increases PD-L1 expression in HPV-positive HNSCC cells in a cyclin-dependent kinase 4 (CDK4) dependent manner [15]. This finding was validated in a murine model, where CDK4 inhibition enhanced the efficacy of anti-PD-L1 therapy [15]. Another study employed a 3D spheroid co-culture of tumor

**Table 4.1** Studies on HNSCC tumor spheroids in the last five years

| First author | Target(s) | Main findings |
|---|---|---|
| Siemer [5] | Cisplatin-resistant HNSCC | Nanoparticle-formulated cisplatin eradicates cisplatin-resistant tumor cells by circumventing the LRRC8A-transport pathway |
| Azharuddin [6] | Multidrug resistance | Profiling drug resistance in cancers with unknown resistance profiles |
| Zhou [8] | DNA repair | PARP inhibition can radiosensitise particularly HPV-negative HNSCC tumor cells to photons and protons |
| Lee [7] | Radiosensitivity | Rapid quantitative assay for evaluating sensitivity to proton beam therapy and drug combinations |
| Allen [34] | MMP9 | Usage of spheroid invasion assays to identify drugs with inhibitory ability of MMP9-mediated invasion |
| Gilardi [9] | H-Ras | Experimental studies in genetically defined HNSCC systems harbouring HRAS mutations supports the rationale for selectively enrolling patients with HRAS-mutant HNSCC in future tipifarnib trials |
| Czerwonka [10] | Notch pathway | Small molecule Notch modulators as interesting therapeutic strategy in selected patients with intact Notch signalling |
| Ghosh [11] | Notch pathway | Low Notch activity associated with higher sensitivity to JAK-selective drugs, Ruxolitinib or Tofacitinib |
| Lamy [35] | Drug uptake | Improvement of cellular uptake of photosensitizer drugs by the use of so-called nanosponges |
| Mhaidly [14] | TME/macrophages | Spheroid model useful for increasing the understanding of the mechanisms underlying cancer cell-macrophage interactions |
| Wu [15] | TME/macrophages | M1 macrophages involved in spatial heterogeneity of PD-L1 expression and tumor invasion; combination therapy of anti-PD-L1 and CDK4 inhibitor as potential treatment for HPV-positive HNSCC identified |
| Nowak [16] | EGFR+ tumor cells | Co-culture of tumor cells with anti-HER1 CAR-NK-92 cells |
| Francois [13] | TME/macrophages | Spheroids can be used to screen immunomodulatory nano-drugs targeting tumor-associated macrophages |
| Eichberger [36] | BMSC-HNSCC interactions | Heterogeneous spheroids that contained both mesenchymal stroma cells and HNSCC cells give insights into mechanisms of bone resorption |
| Sukanya [37] | Bone invasion | Patient-derived tumor spheroids embedded in the osteomatrix can be used for monitoring of the invasion profile and for screening of anti-cancer drugs |
| Ghosh [12] | Tumor stem cells | Characterisation of diversity among human oral stem-like cancer cells |
| Vipparthi [38] | Oral cancer with unknown etiologies | Spheroids from non-tobacco associated tumors allow preclinical investigations of oral cancers caused independent of tobacco usage |

Abbreviations: *BMSC* bone marrow-derived stroma cells, *EGFR* epidermal growth factor receptor, *HPV* human papilloma virus, *TME* tumor microenvironment

cells and chimeric antigen receptor (CAR)-NK cells targeting EGFR to assess the potential of CAR-NK cell therapy in HNSCC [16]. They demonstrated that these models are not only suitable for assessing the tumor cell killing activity of CAR-NK cells but also for elucidating mechanisms of resistance against immune cell attacks [16].

While these examples highlight the potential of spheroids in discovery of novel therapeutic strategies, the lessons learned from these studies stress the importance of standardizing assays and scaling up processes. This is critical to accelerate the transition from preclinical research to clinical applications. For a comprehensive general review on current spheroid generation technologies, I would like to refer the reader to the excellent paper by Liu et al. [17]. Their review comprehensively outlines the diverse manufacturing capabilities of spheroid technologies, including hanging drop methods, agitation-based systems, microfluidics, and bio-printing. Additionally, the authors highlight the potential applications and future directions of spheroid technology, emphasizing its significance in drug discovery, disease modelling, and tissue engineering. They also discuss key challenges and opportunities for advancing the field, such as improving automation, standardisation, and integrating advanced materials and biofabrication strategies.

## Tumor Organoids

The first report on HNSCC organoids was published in 1991 when Köpf-Maier & Zimmermann [18] described a new cell culture protocol that allows human tumor tissues to proliferate and differentiate *in vitro* into organoids. Already in these initial studies it was well recognized that HNSCC organoids forming under organoid-specific culture conditions exhibited cytological and histological characteristics typical of their original tissue [18]. As opposed to spheroids, which do not require a scaffolding to form 3D cultures, organoids are complex clusters of organ-specific cells, which self-assemble into microscopic versions of parent organs only when given a scaffolding extracellular environment (e.g., Matrigel). Another difference is that each organoid in the ex vivo culture, derived from patient tumor tissue, represents a monoclonal structure originating from one single tumor (stem) cell whereas patient-derived spheroids are oligo/polyclonal cell clusters. Sato and coworkers provided the first experimental proof that individual adult intestinal stem cells have the capability to generate 3D intestinal organoids in Matrigel [19]. They showed that these organoids autonomously organise and undergo differentiation into crypt-villus structures even without a mesenchymal niche.

Tanaka and colleagues published the first significant series of organoids from head and neck cancer patients in 2018 [20]. Subsequent studies [21, 22], along with our own unpublished data from a cohort of 186 patients (Fisch A.S. et al., submitted for publication, April 2024) confirmed successful establishment in approximately one-third of cases when primary tumor tissue specimens from diagnostic biopsies or

surgical resections were used for organoid establishment. Over the past five years, tumor organoids have been utilized in HNSCC research across diverse contexts (see Table 4.2). These applications include drug and radio sensitivity screening, elucidation of the role of gene amplifications in oral cancer genesis [23], characterization of tumor stem cells [24], exploration of signalling pathways involved in the stemness phenotype [25], and the development of (molecular) drugs [26, 27] in combination with predictive biomarkers of their effectiveness [28, 29]. The majority of studies, however, have focused on developing patient-derived organoids (PDOs) as

**Table 4.2** Studies on HNSCC tumor organoids in the last five years

| First author | Target(s) | Main findings |
|---|---|---|
| Lucky [39] | Radiosensitivity | Patient-derived nasopharyngeal cancer organoids for optimizing radiation dose |
| Wang [27] | Drug development | Establishment of a patient-derived organoid model and living biobank for drug development in nasopharyngeal carcinoma |
| Yoon [29] | Drug development | hTERT as a potential drug target in high-risk oral squamous cell carcinoma |
| Driehuis [26] | Drug development | Patient-derived organoids recapitulate EGFR expression levels of patient tumor tissues and are responsive to EGFR-targeted photodynamic therapy |
| Salahudeen [23] | Gene amplifications | Functional screening of oncogenes affected by gene amplification in organoid models for deciphering their role in tumor genesis |
| Johansson [24] | Tumor stem cells | Characterisation of cancer stem-like cell populations within epithelial organoids of the tongue |
| Shimonosono [25] | Tumor stem cells | Alcohol metabolism enriches HNSCC stem cells that are resistant to oxidative stress via autophagy |
| Driehuis [22] | Drug and radiosensitivity screens | Oral mucosal organoids might serve as a potential platform for personalized therapy |
| Perréard [32] | Individualized treatment selection | Establishment of tumor organoids to assess tumor response to innovative therapies |
| Ding [31] | Individualized treatment selection | Molecular landscape and subtype-specific therapeutic response of nasopharyngeal carcinoma cells can be revealed by integrative pharmacogenomics |
| Millen [21] | Individualized treatment selection | Patient-derived organoids allow treatment stratification and serve as a tool for biomarker validation and identification |
| Choi [30] | Individualized treatment selection | Clonal evolution of long-term expanding HNSCC might impact the accuracy of personalized therapeutic screening results |
| De Kort [28] | Biomarker development | p-mTOR, p-ERK and PTEN expression in tumor biopsies and organoids identified as predictive biomarkers |

Abbreviation: *HPV* human papillomavirus

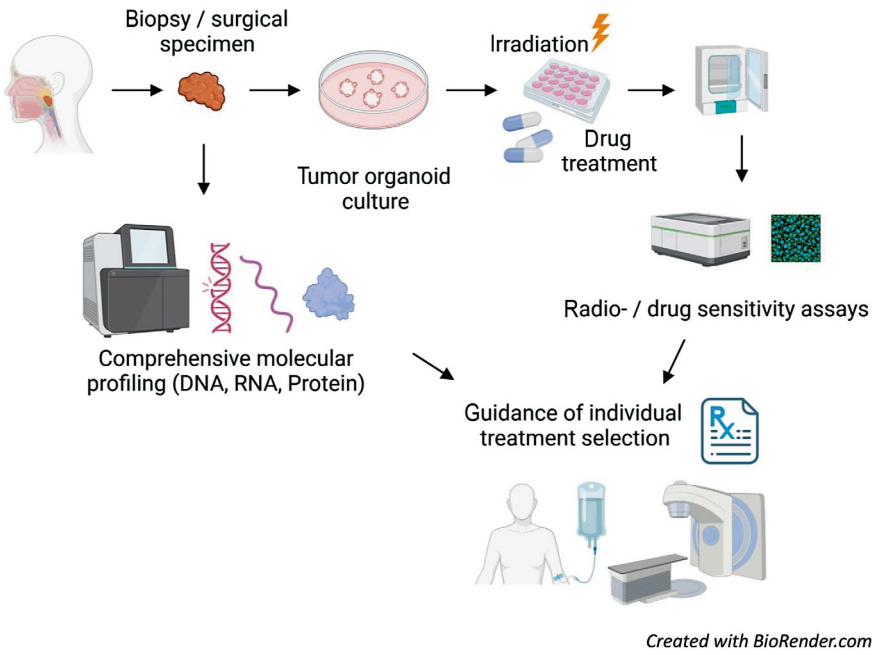


**Fig. 4.1** Functional precision oncology workflow based on tumor organoids for guidance of individual treatment decisions

a platform for individualized treatment selection [21, 22, 30–32]. The inclusion of such a functional screening platform in a diagnostic workflow, as schematically outlined in Fig. 4.1, holds the potential to revolutionize current precision oncology practices, which predominantly rely on comprehensive genomic profiling of tumors. Functional assays in organoids may be employed to assess how tumor cells respond to radiation, drugs, or treatment combinations in a laboratory setting. Supported by comprehensive characterisation of the tumor's molecular profile and functional behavior, clinicians can select targeted therapies or personalized treatment regimens that are more likely to be effective for the individual patient, potentially improving treatment outcomes and minimizing adverse effects.

Insufficient rates of organoid engraftment undoubtedly present a significant challenge to the clinical incorporation of PDOs for guiding individual treatment strategies. Previously reported success rates of 28% to 35% [20, 22] would thus significantly impair the clinical applications of organoids for precision oncology in HNSCC. However, Millen and colleagues [21], along with observations from our extensive patient sample series mentioned above, have demonstrated that success rates could be increased from ~30% to 80% by implementing an initial quality check of the tumor cell content in the original tissue specimen and conducting a re-sampling procedure in cases of insufficiently high tumor cell content.

The time elapsed between sample collection and the availability of *ex vivo* screening results is another crucial factor determining the clinical feasibility of PDO-based functional screens. Recent advancements in commercial automation equipment can handle labour-intensive steps of the screening process, such as cell seeding, with preliminary findings suggesting enhanced reproducibility when performed by machines. Scaling down culture sizes and increasing density, from 96- to 384-well plates, can enhance efficiency and reduce costs. Moreover, the use of 384-well plates offers the added benefit of supporting additional quality control through built-in positive control wells. Although organoids are in principle amenable to high-throughput screenings, progress has been impeded by technical limitations and the extensive manipulations required by current methodologies. Recently, a miniaturized method was introduced that uses a simplified geometry by seeding cells around the rim of the wells (mini-rings) [33]. This advancement enables high-throughput screenings of organoids in a format compatible with automation. Leveraging this automated screening platform, personalized responses of PDOs to a panel of 240 kinase inhibitors were analysed [33]. Crucially, results were obtainable within a week from surgery, aligning with the timeline for therapeutic decision-making.

## Conclusions

Tumor spheroids and organoids have received increasing research interest in the past decades and are currently used in wide applications such as tumor cell biology, drug screening and biomarker development. Various technologies are being used for spheroid / organoid generation, which have their own advantages and limitations. In general, current cell-based assays are highly manual and have slow turnaround time with limited process control, throughput and scalability. To meet increasing demands for cell-based assay applications, future technological development has to be made to improve current technologies to make large-scale, consistent, and repeatable manufacturing of spheroid and organoids possible. Such development efforts should focus on areas of liquid handling, transfer, characterisation, process standardization and process scalability.

Integration of spheroids and organoids in individual treatment selection could significantly contribute to treatment optimization in HNSCC. Early investigations have identified great potential but also barriers hindering the clinical integration of these personalized tumor models, yet advancements like microfluidics technology offer potential solutions. Nonetheless, further prospective clinical trials are essential to validate the utility and feasibility of personalized functional assays in clinical practice. These trials will be pivotal in realising the full potential of personalized tumor models for individualized cancer care.

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# Chapter 5
# Is the ESCAT Ranking of Support in Head and Neck Cancer Management?


**Maud Kamal and Constance Lamy**

## ESMO Scale of Clinical Actionability in Cancer (ESCAT)

Precision oncology has transformed cancer treatment paradigms by tailoring therapies to individual patients based on the molecular characteristics of their tumors. With the advent of high-throughput genomic sequencing technologies, identifying actionable mutations has become increasingly common. However, not all molecular alterations have equal clinical significance, and prioritizing them for therapeutic decision-making is crucial. In response to this challenge, the European Society for Medical Oncology (ESMO) introduced the ESMO Scale of Clinical Actionability in Cancer (ESCAT), providing a standardized framework for assessing the clinical relevance of molecular alterations in cancer patients. ESCAT classifies alterations into six tiers, ranging from level Tier I (highest clinical utility) to Tier X (no clinical utility). The scale considers factors such as the presence of approved therapies targeting the alteration, the strength of evidence from clinical trials, and the availability of predictive biomarkers for treatment response [1].

- Tier I (Highest Clinical Utility-Ready for Routine Use): alterations falling into this category have strong clinical evidence supporting their predictive or prognostic significance. They are associated with approved targeted therapies or have demonstrated clinical benefit in well-designed clinical trials. Examples include *MET* mutation (exon 14-skipping) in lung cancer targeted by tepotinib, an ALK, MET, ROS1 inhibitor [2]. Clinical trials evaluating TRK inhibitors such as larotrectinib and entrectinib have demonstrated remarkable responses in patients with *NTRK* fusion-positive cancers classifying *NTRK* fusions as Tier I in pan cancer [3].

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- Tier II (Potential Clinical Utility-Investigational Targets): alterations categorized as Tier II possess promising clinical evidence but may lack conclusive data from large-scale clinical trials. They are often associated with investigational therapies or off-label use of approved drugs based on preclinical or early-phase clinical data. Tier II alterations may include less common mutations or alterations in genes with emerging therapeutic implications. Taking the example of tepotinib and *MET* alterations, *MET* amplifications became Tier II in lung cancer [2].
- Tier III (Limited Clinical Utility-Hypothetical Targets): alterations in this category have limited clinical evidence supporting their therapeutic relevance. They may be associated with targeted therapies in preclinical models or early-phase clinical trials, but robust clinical validation is lacking. Tier III alterations require further investigation to establish their clinical utility and may be considered for enrollment in clinical trials or research studies. Examples include *MET* mutation (exon 14-skipping) in head and neck cancer (HNC) targeted by tepotinib [2].
- Tier IV (No Clinical Utility): alterations categorized as Tier 4 lack clinical evidence supporting their relevance to cancer treatment. They may represent benign variants, germline polymorphisms, or alterations with no known therapeutic implications. Level IV alterations do not guide treatment decisions and are typically considered incidental findings in genomic profiling.
- Tiers V: alterations to be considered in combination development. Drug-target match associated with objective responses without clinically meaningful benefit.
- Tier X; alterations should not be taken into account for clinical decisions due to lack of evidence of actionability

## ESCAT in Head and Neck Cancers

Head and neck cancer encompasses squamous cell carcinoma (SCC) and other rare cancers such as adenoid cystic carcinoma (ACC). SCC, the most common type, arises from squamous cells in the mouth, throat, larynx, or nose, often linked to tobacco, alcohol, HPV infection, or certain exposures. Head and neck squamous cell carcinoma (HNSCC) ranks as the seventh most common cancer worldwide [4]. While localized HNSCC can often be treated with surgery and/or (chemo)radiation, the prognosis remains dismal. Current treatment approaches primarily focus on tumor location and stage, disregarding underlying causes such as HPV infection or tobacco and alcohol use, despite HPV being associated with a more favorable prognosis [5]. Pembrolizumab, an immune checkpoint inhibitor, has become a standard first-line treatment for recurrent or metastatic HNSCC, often combined with platinum-based chemotherapy [6]. Targeted therapies, particularly those aimed at the epidermal growth factor receptor (EGFR), have shown limited success, underscoring the need for novel treatment strategies. Next-generation sequencing (NGS) technologies have identified actionable molecular alterations in HNSCC, offering potential new therapeutic avenues. However, relatively few clinical trials have been conducted in specific HNSCC patient populations [7].

Adenoid cystic carcinoma (ACC) is a rare and often challenging cancer, known for its resistance to treatment and tendency to metastasize. While it typically originates in salivary glands, ACC can also develop in the trachea, lungs, breasts, and other areas. Treatment usually involves surgery followed by radiation therapy, but due to its propensity for perineural invasion and distant spread, local recurrence and late distant metastases are common, occurring in over 50% of cases over time [8]. Unfortunately, relapsed ACC tumors are typically incurable as current systemic treatments have shown limited effectiveness. Consequently, the long-term prognosis remains poor, with overall survival rates ranging from 23% to 40% [9].

The current manuscript gives an overview of the potential clinical impact of matching targetable molecular alterations in patients with HN cancers according to ESCAT with a focus on Head and neck squamous cell carcinoma (HNSCC) and adenoid cystic carcinoma (ACC). Preliminary unpublished data from Institut Curie molecular tumor board (MTB) are also reported.

## ESCAT in Head and Neck Squamous Cell Carcinoma (HNSCC [10])

### *Molecular Alterations of ESCAT Tier I/II in HNSCC*

Considering the tissue-agnostic approval of pembrolizumab for patients with tumors that have high mutational burden (TMB-high) and microsatellite instability (MSI) by the FDA, we ranked TMB-high and MSI as Tiers I in HNSCC. Similarly, *NTRK* fusions with a frequency of 0.2% [10] are ranked Tier I. *HRAS* is the most frequently altered gene among the three *RAS* family in HNSCC with a frequency of 6.3%. *HRAS*-activating mutations are ranked Tier II based on the high antitumor activity of tipifarnib in a phase II study in advanced tumors with *HRAS*-activating mutations [11, 12].

*CDKN2A*-inactivating alterations were reported in 53.8% of patients with HNSCC [10]. Palbociclib, a selective CDK4/6 inhibitor, was evaluated in combination with cetuximab in the PALATINUS trial (NCT02499120). Based on a retrospective analysis of a molecularly defined subgroup in the PALATINUS trial, *CDKN2A*-inactivating alterations were classified as tier II [13].

*EGFR* is commonly amplified in HNSCC (10.7% of patients). A retrospective exploratory biomarker analysis based on the LUX-Head & Neck 1 trial showed a significant improvement of median PFS in the subgroup of patients with an *EGFR* amplification, classifying *EGFR* amplification as tier II [14]. Despite an improved overall survival (OS) in randomized trials in HNSCC [15] cetuximab was excluded from the classification, since EGFR amplifications were not shown to predict the efficacy of cetuximab in combination with chemotherapy [16].

### *Molecular Alterations of ESCAT Tier III in HNSCC*

*PIK3CA*-activating mutations are reported in 12.9% of HNSCC. In patients with metastatic breast cancer, an improved OS was reported in patients with E542K, E545K/A, and H1047R/L hotspot–activating mutations in *PIK3CA* with the alpha-selective PI3K inhibitor alpelisib [17]. Based on the latter trial, E542K, E545K/A, and H1047R/L hotspot–activating mutations in *PIK3CA* are classified as tier III in HNSCC.

Promising antitumor activity was reported with the pan-AKT inhibitors capivasertib and ipatasertib in advanced breast and prostate cancers in the subgroup of patients with a *PTEN* alteration. We therefore classified PTEN-inactivating alterations in tier III in HNSCC [18].

Alterations in tyrosine kinase receptors (*ERBB2*, *FGFR1*, *FGFR3*, *MET*) or DNA repair pathway genes (*BRCA1*, *BRCA2*, *POLE*) are classified as Tier III since clinical benefit was demonstrated in other tumor types [10].

### *Molecular Alterations of ESCAT Tier IV/V/X in HNSCC*

The use of the other tiers is not straightforward when it comes to their application in treatment decision. The main alterations in these tiers are summarized in Marret et al., 2021 [10]. For example, *IGF1R* amplifications are considered as Tier IV based on preclinical evidence in HNSCC; T53 inactivating alterations could be considered as Tier V while CCND1 amplifications are classified as tier X due to lack of evidence.

## ESCAT in Adenoid Cystic Carcinoma (ACC)

The most frequent mutations in ACCs are translocations affecting *MYB* or *MYBL1*, which are currently not targetable. As a result, the majority of potentially treatable mutations in ACCs are tyrosine kinases. Approximately 40.3% of recurrent or metastatic (R/M) ACC tumors contain mutations in genes (*CSF1R*, *EGFR*, *ERBB2-4*, *FGFR1-3*, *KDR*, *KIT*, *PDGFRA-B*...) that could be targeted with existing kinase inhibitors, however clinical evidence remains to be demonstrated in ACC. Consequently, alterations in *CSF1R, EGFR, ERBB2-4, FGFR1-3, KDR, KIT, PDGFRA-B, SRC* are classified as Tier III in ACC [19].

## Use and Impact of ESCAT in Molecular Tumor Board (MTB)

In the era of precision oncology, where targeted therapies are administered based on validated MA and next-generation sequencing allows for timely patient screening within clinical practice, MTB orchestrates and integrates molecular and clinical information to inform treatment decisions. Here, we present data on patients with HNSCC and ACC screened between 2018 and 2023 at Institut Curie's MTB (unpublished data). Patients included in Institut Curie MTB are adult patients (>18 years) with advanced cancer in first-line failure or rare cancers, histologically proven malignant tumor with an available pathological report, unresectable and/or metastatic disease, at least one tumor lesion amenable to biopsy or available archival tumor material. Molecular alterations identified in HNSCC patients are depicted in Fig. 5.1. Among the 45 HNSCC patients discussed in Institut Curie MTB, only three (9%) received targeted therapy and were consequently classified according to ESCAT guidelines. Stable disease (SD) (PFS of 8 months) was observed in one HNSCC patient with an *FGFR3* mutation (Tier III) treated with erdafitinib (Table 5.1). Additionally, one of the 17 ACC patients (8%) received durvalumab + tremelimumab based on high TMB with SD and a PFS of 15 months (Fig. 5.2; Table 5.1). Despite the limited dataset and patient numbers, which restrict the ability to draw definitive conclusions, there is a clear indication that screening HNC patients at MTBs should be encouraged to accumulate real-world evidence data. This approach enables the gathering of valuable insights and outcomes from a broader patient population over time. By encouraging such screening practices, healthcare professionals can enhance their understanding of treatment responses, refine therapeutic strategies, and ultimately improve patient outcomes in the long term.

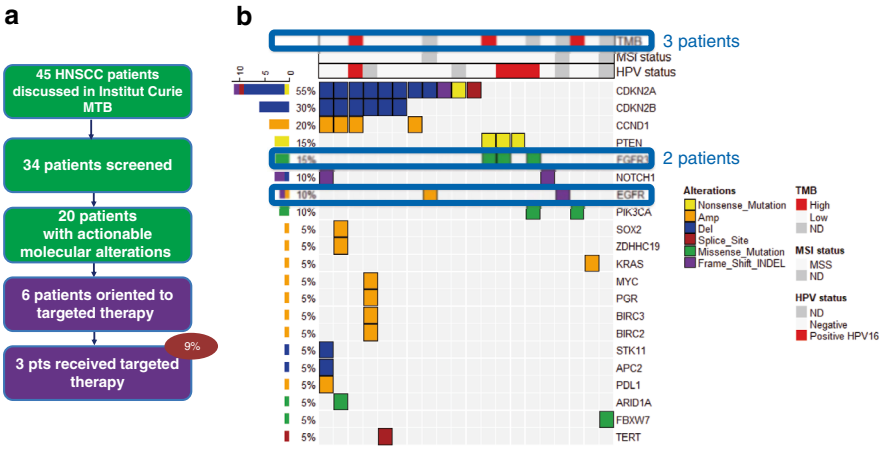


**Fig. 5.1** (**a**) Head and neck squamous cell carcinoma (HNSCC) patients screened in Institut Curie Molecular Tumor Board (MTB). (**b**) Oncoprint highlighting actionable alterations screened via Institut Curie MTB. TMB: Tumor Mutational Burden; MSI: Microsatellite Instable; MSS: Microsatellite Stable; ND: not done; Amp: amplification; Del: Deletion

**Table 5.1** HNSCC and ACC patients treated with matched therapy in Institut Curie Molecular Tumor Board (MTB)

| ESCAT TIER | Molecular Alteration (MA) | Matched therapy | ORR/PFS |
|---|---|---|---|
| HNSCC | | | |
| I | TMB-high | Anti-PD1 | PD |
| III | *FGFR3* mutation (c.746C>G / p.(Ser249Cys) | FGFR3 inhibitor (erdafitinib) | SD (PFS = 8 months) |
| III | *FGFR3* mutation (c.746C>G / p.(Ser249Cys) | FGFR3 inhibitor (erdafitinib) | PD |
| ACC | | | |
| I | TMB-high | Anti-PDL1 + anti-CTLA-4 (durvalumab + tremelimumab) | SD (PFS = 15 months) |

*TMB* tumor mutational burden, *ORR* objective response rate, *PD* progressive disease, *SD* stable disease, *PFS* progression free survival

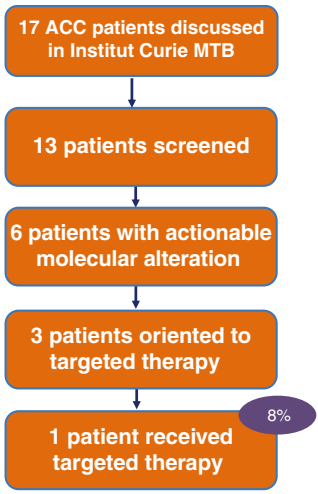


**Fig. 5.2** Adenoid cystic carcinoma (ACC) patients screened in Institut Curie Molecular Tumor Board (MTB)

## Conclusions

The impact of ESCAT on the management of patients with HNC, particularly in rare cases such as ACC, is yet to be fully demonstrated. While over 30 genes are considered actionable in HNSCC, only a limited number have been classified as Tiers I/II, indicating a higher level of clinical evidence. In ACC, approximately 40% of MA are potentially targetable by tyrosine kinase inhibitors, but the clinical evidence supporting this remains to be established.

The primary indication for employing the ESCAT classification in HNC is therefore for patients undergoing genomic profiling through MTB. Within this context, it is crucial to encourage the enrolment of HNC patients in clinical trials that utilize drugs matched to their molecular alterations.

In summary, ESCAT serves as a valuable tool for integrating genomic information into clinical decision-making and advancing precision oncology. By categorizing molecular alterations based on their clinical relevance, this scale empowers oncologists to customize treatment strategies for individual patients, ultimately improving outcomes in cancer care.

ESCAT classification needs however to be updated to overcome several challenges including validating biomarkers, integrating real-world evidence, and addressing disparities in access to targeted therapies. Moreover, as the field of cancer genomics evolves rapidly, ESCAT must adapt to include emerging biomarkers and therapeutic approaches.

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# Chapter 6
# Mechanism of Action and Potential Use of SMAC Mimetics in Head and Neck Squamous Cell Carcinoma (HNSCC)


**Mai K. Bishr and Ben O'Leary**

## Introduction

Evasion of cell death is one of the hallmarks of cancer; a mechanism that promotes cancer cell survival and treatment resistance [1]. For decades, the terms "apoptosis" and "programmed cell death" have been used interchangeably; however, extensive research has revealed the existence of several distinct modes of regulated cell death [2, 3]. Among these, however, apoptosis remains the most studied and well characterised mode of cell death in cancer. It entails a highly orchestrated process of proteolytic cleavage of cell components culminating in cell death via two connected, but distinct, routes: the intrinsic and extrinsic apoptotic pathways [2].

Head and neck squamous cell carcinoma (HNSCC) is the eighth most common malignancy worldwide [4]. Oropharyngeal SCCs are broadly classified into two main subtypes with varying clinical and biological characteristics: human papillomavirus (HPV)-negative and HPV-positive subtypes. The main risk factors for the HPV-negative subtype are smoking and alcohol consumption, while HPV infection is the principal aetiological driver for the HPV-positive subtype [5]. Approximately 60% of HNSCC patients present with locally advanced disease for which multimodality treatment is required for curative intent [6]. Over the past 3 decades, there has been a small improvement in survival outcomes for HNSCC, mainly driven by the good prognostic group of HPV-positive cancers [7, 8], but there remain important open questions regarding the optimal curative treatment of HNSCC, the standard of care paradigm and disease specific clinical outcomes having improved little for decades. Recent progress in the development of SMAC mimetics, drugs that target the pathways that regulate apoptosis, have highlighted these as a potential novel

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therapeutic approach for treatment of HNSCC with curative intent, where locoregional and metastatic relapse are such a major challenge. Targeting apoptosis effectively in combination with agents that already induce cell death, such as radiotherapy and chemotherapy, provide a plausible route to reducing the risk of locoregional recurrence.

## *Recent Approaches to Improve the Standard of Care for Curative Intent in HNSCC*

Management options for HNSCC include surgery and/or (chemo)radiotherapy, depending on the anatomical site, stage, adverse risk factors, performance status, and patient choice. In locally advanced HNSCC, several randomised clinical trials established a significant benefit for the addition of cisplatin chemotherapy with external beam radiotherapy in terms of locoregional control and survival [9–12]. Subsequently, the meta-analysis of chemotherapy in head and neck cancer (MACH-NC) confirmed a 5-year overall survival (OS) benefit of 6.5% for the addition of concomitant chemotherapy with radiation treatment, cementing this as a standard of care for certain patient groups [13]. Since this breakthrough, the treatment landscape for non-metastatic HNSCC has remained relatively unchanged, with efforts to identify patient groups in which platinum chemotherapy could be safely omitted, principally HPV-positive disease. On the contrary, outside low-risk HPV-positive disease, the focus has been on treatment escalation. Novel therapeutic agents, particularly if associated with more tolerable side effect profile than platinum chemotherapy, could potentially have a role to play in both of these scenarios.

There has been growing interest in exploring treatment de-escalation strategies in HPV-positive oropharyngeal SCC owing to its favourable prognosis and prevalence of younger patients for whom the prospect of reducing treatment-related toxicity and long-term morbidity, without compromising oncological outcomes, is attractive. This has been explored in several trials using cetuximab, a monoclonal antibody against the epidermal growth factor receptor (EGFR). The combination of radiotherapy/cetuximab was compared to radiotherapy/3-weekly cisplatin in the non-inferiority RTOG 1016 trial and the De-ESCALaTE study in patients with HPV-positive oropharyngeal carcinoma [14, 15]. The primary endpoint was not met in both studies; in the RTOG 1016 study, the estimated 5-year OS for the cetuximab group was 77.9% (95% CI 73.4–82.5) compared to 84.6% (95% CI 80.6–88.6) in the cisplatin group [14], and in the De-ESCALaTE study, there was no difference in the rate of severe toxicities between both regimens at 24 months with detrimental 2-year OS in the arm with cetuximab [15]. Similar observations were also noted in the TROG 12.01 study comparing radiotherapy/cetuximab to radiotherapy/weekly cisplatin [16]. Collectively, these results strongly demonstrate that the strategy of substituting cisplatin with cetuximab has a detrimental effect on patient outcomes and that this is not a viable strategy, particularly in HPV-positive oropharyngeal carcinoma. Alternative treatment de-escalation strategies are currently being

explored in the HPV-positive patient cohort, including surgical management to spare the need for (chemo)radiation for some patients and dose de-escalation in adjuvant radiotherapy. Data from small cohorts and phase II trials have supported the safety of these approaches [17, 18], but questions remain over patient selection and comparative toxicity profiles [19, 20]. Results from the ongoing PATHOS clinical trial (NCT02215265) are expected to help address these questions [21].

Despite aggressive treatment, locoregional and metastatic relapse remain a significant challenge, occurring in around 50% of patients treated for locally advanced disease within 2 years, less so in HPV-positive oropharyngeal tumours [22]. Only a minority of such patients would be eligible for radical intent treatment with salvage surgery and/or radiotherapy and treated with curative intent [23]. Recent attempts to improve locoregional control with dose-escalated radiotherapy have been unsuccessful. Anti-programmed cell death protein 1 (PD-1) immune checkpoint inhibitors revolutionised the treatment landscape of relapsed/metastatic disease, but only a small proportion of patients experience durable responses [23–27]. In the KEYNOTE-048 study, pembrolizumab/chemotherapy combination conferred an OS benefit compared to cetuximab/chemotherapy, regardless of PD-L1 status (HR 0.71, 95% CI 0.59–0.85) [24, 25]. In the same trial, pembrolizumab monotherapy also improved OS compared to cetuximab/chemotherapy, but only in PD-L1 positive tumours (defined as combined positive score (CPS) $\geq$1) (HR 0.74, 95% CI 0.61–0.89) [24, 25]. In patients who progressed on or after platinum-based treatment, nivolumab improved OS compared to chemotherapy (HR 0.68, 95% CI 0.54–0.86), regardless of PD-L1 status, as shown in the Checkmate-141 study [26, 27]. Despite the success of immunotherapy in the relapsed/metastatic setting, several studies failed to demonstrate improved outcomes for immunotherapy in combination with standard of care treatment in the locally advanced disease, with a variety of immune checkpoint inhibitors in different high-risk patient populations [28–30]. These findings highlight an unmet need to improve clinical outcomes in this setting; one plausible direction is to target treatment resistance mechanisms including evasion of apoptosis.

## *Role of Apoptosis in HNSCC*

Apoptosis is a strictly regulated process of cell death which is fundamental for maintaining tissue homeostasis, and its disruption is implicated in a wide variety of malignancies. It is characterised by key morphological features, including nuclear condensation and fragmentation, blebbing of the cell membrane, formation of apoptotic bodies, and degradation of most of the cellular macromolecules, including DNA [31, 32]. These characteristic hallmarks facilitate the detection of apoptosis *in vivo*, however, apoptotic cells are usually promptly engulfed and degraded before acquiring the full apoptotic phenotype, which suggests that the incidence of apoptosis is generally underestimated [32]. The process of apoptosis is orchestrated by a series of signalling cascades, under the influence of three critical components:

caspases, inhibitor of apoptosis proteins (IAP), and IAP antagonists [31]. Caspases are proteolytic enzymes, present in an inactive form as pro-caspases, and are broadly classified into initiators and effectors (also known as executioners) [33]. Upon receipt of specific death-inducing stimuli, the initiator caspases (capsase-2, −8, −9 and − 10) are activated and initiate the respective apoptotic pathway, while the effector caspases (caspase-3, −6 and − 7) act directly on specific cellular substrates leading to their degradation [33]. This process is executed through two pathways, the intrinsic, which responds to intracellular damage and stress signalling, and the extrinsic, which provides a signalling link with the extra-cellular milieu (Fig. 6.1) [33].

## The Intrinsic (Mitochondrial) Pathway

The intrinsic apoptotic pathway is activated by non-receptor-mediated DNA-damaging stimuli, such as chemotherapy and radiotherapy, inducing mitochondrial outer membrane permeabilization (MOMP) with subsequent release of sequestered pro-apoptotic proteins into the cytoplasm [34]. These proteins include cytochrome c, second mitochondria-derived activator of caspases (SMAC)/direct IAP-binding protein with low pI (Diablo), and Omi stress-regulated endoprotease/High

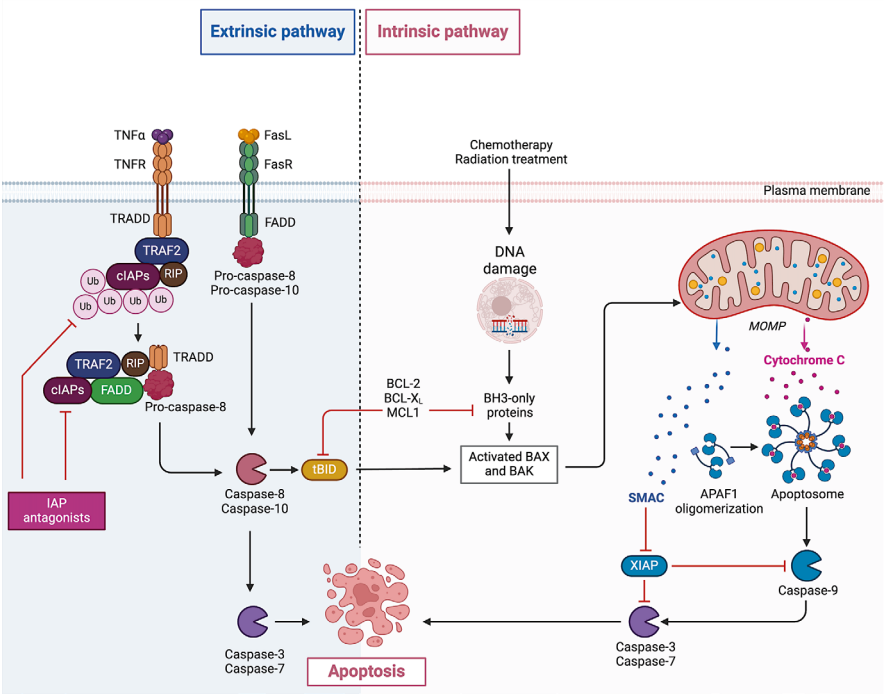


**Fig. 6.1** Schematic diagram of the intrinsic and extrinsic apoptotic pathways. Created with BioRender.com

temperature requirement protein A2 (Omi/HtrA2) [34]. This cascade of events leads to activation of caspases −9, −3, and − 7 with execution of apoptosis [34]. Additionally, MOMP can induce caspase-independent cell death (CICD), separate to the canonical apoptotic signalling pathway, via stimulating NF-κB activity and the cGAS/STING pathway [35]. The intrinsic pathway is predominantly regulated by intricate interactions between the BCL-2 subfamilies: the anti-apoptotic subfamily (BCL-2, BCL-XL and MCL1), the pro-apoptotic subfamily (BIM, PUMA and BID), and the death effectors (BAK and BAX) [36].

Within the context of HPV infection, the viral oncoprotein E6 is known to induce degradation of p53 and the death effector, BAK, leading to inhibition of caspase-mediated apoptosis [37]. In contrast to HPV-positive cases, HPV-negative HNSCC harbours *TP53* mutations in more than 80% of cases [38]. Thus, it is unsurprising that evasion of cell death is observed in these tumours owing to the cardinal role of *TP53* in regulating the apoptotic machinery [37, 39].

### The Extrinsic (Death Receptor) Pathway

Activation of the extrinsic apoptotic pathway is initiated by binding of death ligands (such as tumour necrosis factor (TNF), FasL and TRAIL) with their respective death receptors of the TNF superfamily (for example, tumour necrosis factor receptor (TNFR) and FasR) [34]. This results in the death-inducing signalling complex (DISC) formation and activation of caspases −8 and − 10 leading to either activation of the executioner caspases (−3 and − 7) or BID activation with convergence on the mitochondrial apoptotic pathway [34].

Several genes involved in this pathway are altered in HNSCC, including amplification of Fas-associated death domain (*FADD)*, which regulates DISC formation; mutation of *CASP8*, responsible for pro-caspase 8 production; as well as *TNFSF10*, which encodes TRAIL [37]. In HPV-positive HNSCC, HPV proteins seem to modulate the apoptotic pathway promoting survival of the infected cells. HPV16 E5 was shown to hinder DISC formation and TRAIL apoptotic signalling pathway [40], and E6 is known to mediate degradation of pro-caspase 8, TNFR, and FADD [41]. Moreover, E6 and E7 lead to upregulation of the inhibitor of apoptosis gene, cellular inhibitor of apoptosis protein 2 (c-IAP2) resulting in apoptosis resistance [42].

### Inhibitor of Apoptosis Proteins (IAPs)

IAPs are a family of endogenous negative regulators of apoptotic pathways constituting of 8 proteins in humans: X-linked IAP (XIAP), survivin, cellular IAP 1 and 2 (cIAP1/2), neuronal apoptosis inhibitory protein (NAIP), melanoma IAP, IAP-like protein 2 (ILP-2), and apollon [43, 44]. The most studied and best understood of these are XIAP and cIAP1/2. XIAP can directly bind and inhibit both initiator and effector caspases [45], while cIAPs inhibit the formation of pro-apoptotic

complexes within the extrinsic pathway and suppress the non-canonical NF-κB signalling pathway, increasing TNFα [46]. Survivin has been shown to inhibit caspase 9 activity as well as caspase-independent apoptosis via regulating apoptosis-inducing factor (AIF) [47]. IAPs also play an important role in the regulation of several other signalling pathways, including p38 mitogen-activated protein kinases (MAPK), transforming growth factor beta (TGF-β), innate and adaptive immune signalling [46, 48–50]. XIAP was found to co-localise with TGF-β type I receptor (TβRI) and activate the transcription of TGF-β-responsive promoters [49], while cIAPs associate with TNFR2 and TNFR-associated factors (TRAFs) as signal transduction intermediates in activating the c-Jun N-terminal kinase (JNK) and MAPK pathways [48]. IAP activity is, in turn, regulated by several feedback mechanisms involving pro-apoptotic proteins that act as IAP antagonists, such as Smac/Diablo and Omi/HtrA2 [46]. The physical interaction between Smac and IAPs leads to IAP neutralisation, release of negative regulation on caspases and activation of the apoptotic signal, thereby inhibiting apoptosis inhibitors [51, 52].

There is a variety of evidence indicating that IAP overexpression in cancers could be linked to treatment resistance and poor prognosis in HNSCC. In locally advanced HNSCC, XIAP overexpression was associated with cisplatin resistance and adverse clinical outcomes [53]. In oral cavity SCC, nuclear cIAP1 expression, and to a lesser extent cytoplasmic expression, was correlated with advanced stage and propensity for lymph node metastasis [54] and cIAP2 overexpression was associated with resistance to chemoradiation [55]. Survivin overexpression was also shown to be a poor prognostic factor in HNSCC, regardless of p16 status, especially in patients receiving radiation treatment [56–58]. These observations render IAPs promising therapeutic targets and open new avenues for drug discovery and development.

## *SMAC Mimetics to Target IAPs*

The physical interaction between SMAC and the IAPs invited the pursuit of small molecule antagonists since mapping the IAP-SMAC binding site topology [44]. IAPs are characterised by the presence of the Baculovirus IAP Repeat (BIR) domain at the N-terminal and the Really Interesting New Gene (RING) domain at the C-terminal [34]. The former is composed of 1–3 motifs of about 70 amino acids, while the latter has a characteristic E3-ubiquitin ligase activity [59]. The BIR domain displays distinct protein binding properties including a hydrophobic groove which binds conserved IAP binding motifs (IBMs) on caspases and IAP antagonists [60]. SMAC has also been shown to have selective binding affinity to BIR in an IBM-dependent fashion [61]. Of note, the intrinsic binding affinity varies between different IAP family members due to subtle changes in their respective BIR domains resulting in target protein selectivity [60].

## The Development of SMAC Mimetics

Technological advancements in the field of drug discovery have paved the way to the development of SMAC-mimicking compounds with improved IAP-binding affinities [62]. SMAC mimetics are designed to exhibit non-peptidic structural elements that closely resemble the N-terminal IAP-binding site of the naturally occurring endogenous SMAC compound [44]. The binding of SMAC mimetics with XIAP results in caspase activation [63], while their binding with cIAPs enhances the latter's E3 ligase activity leading to their autoubiquitination and proteasomal degradation [64]. SMAC mimetics-induced cIAP depletion results in accumulation of nuclear factor-κB-inducing kinase (NIK), activation of non-canonical NF-κB signalling and TNF-α upregulation [44, 65]. In an autocrine/paracrine regulatory process, TNF-α then triggers the extrinsic apoptotic pathway [65].

In the early phase of their development, SMAC mimetics were designed as monovalent compounds but subsequently, bivalent compounds were found to display higher binding affinity to IAPs [66]. The new class of bivalent antagonists, consisting of two chemically linked SMAC mimetics, promoted the dimerization of BIR2-BIR3 domains of c-IAP1, simultaneously engaged the BIR2 and BIR3 domains of XIAP, and demonstrated significant high potency in cell death assays [44, 65, 66]. Moreover, bivalent antagonists were shown to be more effective in inhibiting TNF-mediated NF-κB signalling than monovalent compounds and are considered to be the drug of choice for targeting this signalling pathway [67]. Following treatment with monovalent antagonists, high levels of residual TRAF2-associated cIAP1 were noted, reflecting the lack of cIAP1 E3-ubiquitin ligase complex formation which coordinates TRAF2-associated cIAP1 degradation [67]. Collectively, these discoveries led to subsequent expansion in the repertoire of IAP targeting therapeutics in solid and haematological malignancies.

## Pre-clinical and Translational Studies of SMAC Mimetics in HNSCC

Xevinapant, formerly known as Debio 1143 or AT-406, is a first-in-class oral IAP inhibitor and the most clinically advanced SMAC mimetic in HNSCC to date. Its IAP inhibitory function is exerted by blocking XIAP and cIAP1/2, thereby abolishing their negative regulatory effect on cancer cell apoptosis and restoring sensitivity to anti-cancer treatments including chemotherapy and radiation treatment [52]. Preclinical data in different models have at times highlighted how the various contributions of the different mechanisms of SMAC mimetics may be context dependent. In a study on HNSCC cell lines, xevinapant was shown to enhance the intrinsic cellular radiation sensitivity in a time-dependent fashion; this effect was only noted when xevinapant was administered concurrently with radiation treatment and thereafter in a continuous dosing schedule or started 24 h later and continued for 10 days, but not when xevinapant was administered concurrently with radiotherapy for only 6 or

24 h post-irradiation, highlighting the importance of xevinapant timing in relation to radiation treatment [68]. In the same study using mouse xenograft models, its radio-sensitizing effect was mediated by caspase 3 activation and increased TNF-α levels, with more pronounced activity when xevinapant was delivered over 3 weeks (during and after radiotherapy), rather than 2 weeks (only during radiotherapy) [68]. Another study on HNSCC cell lines and surgically resected tumour samples from patients with HNSCC revealed that single agent xevinapant had limited anti-proliferative activity, but this was potentiated with the addition of TRAIL or TNF-α [69]. Additionally, the combination of xevinapant with platinum agents (cisplatin or carboplatin) triggered apoptosis, via caspase 3 activation, in patient tumour samples [69]. More recently, extended xevinapant dosing (post concurrent radiotherapy) was demonstrated to be superior to shorter dosing duration with radiotherapy and radiotherapy alone in terms of tumour control and survival in syngeneic tumour-bearing mice [70]. The mechanisms of such benefit may partly be due to modulation of the tumour microenvironment, enhanced antigen-specific T cell response, and suppression of radiotherapy-mediated activation of cancer-associated fibroblasts (CAFs) [70].

Additional SMAC mimetics with demonstrable activity in HNSCC include birinapant (TL32711), tolinapant (ASTX660), and LCL161. Birinapant was shown to sensitize HNSCC lines to cell death when combined with TNFα or TRAIL and inhibit XIAP and c-IAP1/2, while in vivo, it enhanced radiation-induced TNFα expression and delayed tumour growth, more notably in xenograft models with FADD overexpression [71]. Interestingly, CASP8 knockdown, in vitro, enhances birinapant-induced cell death when combined with radiotherapy [72]. In a study investigating combination treatment of birinapant and a WEE1 inhibitor, AZD1775, in HNSCC cell lines, a synergistic anti-tumoural effect was noted with increased sensitivity to TNFα-induced cytotoxicity [73].

IAP inhibition by tolinapant seems to induce a wide variety of immunomodulatory effects. In HNSCC mouse models, tolinapant enhanced radiation-induced immunogenic cell death, stimulated clonal expansion of cytotoxic T lymphocytes, and led to overexpression of major histocompatibility complex (MHC) class I on tumour cells [74]. In another study using syngeneic mouse models, additive tumoricidal effects were noted for tolinapant in combination with radiotherapy, cisplatin, and PD-1 blockade [75]. These combinations resulted in increased levels of cytotoxic T cells and dendritic cells as well as enhanced T-cell cytotoxicity [75]. Intriguingly, the mechanisms of tolinapant-induced anti-tumour effects, when combined with TNFα and TRAIL, seem to partly vary between HPV-positive and -negative HNSCC [76]. In HPV-positive tumours, tolinapant stimulated the restoration of p53 protein expression and pro-apoptotic activity with caspase-dependent apoptosis being the predominant cell death mechanism [76]. In HPV-negative tumours, cell death was mediated by both apoptosis and necroptosis [76].

Differential IAP inhibition activity with HPV status was also shown with LCL161; its combination with radiotherapy resulted in enhanced apoptotic cell death in HPV-negative, but not HPV-positive, HNSCC cell lines [77]. In HPV-negative xenografts, the combination treatment resulted in dramatic tumour

response, cIAP1 degradation, and induction of apoptosis [77]. The authors suggested that this differential effect could be due to the fact that *TP53* and *CDKN2A* are commonly mutated in HPV-negative tumours with reliance on suppressed apoptosis for survival, therefore, they might be more susceptible to radiation treatment following apoptosis re-activation by LCL161 compared to radiation-sensitive HPV-positive tumours [77]. Additionally, on analysing HNSCC tumour samples from The Cancer Genome Atlas (TCGA) by HPV status, they revealed significant upregulation of *BIRC2,* encoding cIAP1*,* and *SURVIVIN* messenger RNA (mRNA) in HPV-negative tumours compared to HPV-positive ones, which could also play a part in their varied response to LCL161, a potent inducer of cIAP1 degradation [77]. LCL161 was also investigated in combination with FasL in HNSCC cell lines and the combined treatment modality significantly enhanced cytotoxicity compared to FasL monotherapy, including the sensitisation of FasL-resistant cell lines [78].

An exploratory window-of-opportunity study investigated the molecular activity of xevinapant in patients with resectable HNSCC where patients received either xevinapant single agent (200 mg/day on days 1–15 +/−2), xevinapant (200 mg/day on days 1–15 +/−2) plus cisplatin (40 mg/m$^2$ days 1 + 8), or cisplatin single agent (40 mg/m$^2$ days 1 + 8) immediately prior to surgery [79]. Following xevinapant administration, there was significant degradation of cIAP1 ($p < 0.05$), but not cIAP2 or XIAP, and increased levels of CD8+ tumour infiltrating lymphocytes, PD-1 and PD-L1 positive immune cells ($p < 0.05$) [79]. Tumour tissue analysis of the xevinapant monotherapy group revealed changes in the expression levels of genes related to the NF-κB signalling pathway [79]. Overall, while SMAC mimetics share a common function of IAP inhibition and apoptosis restoration, it is becoming clear that the intricacies of their mechanism of action and molecular effects vary between different compounds, which warrants comparative mechanistic studies.

## Clinical Studies of SMAC mimetics in HNSCC

Recently, there has been a surge in the number of clinical trials investigating the role of SMAC mimetics in several cancer types, including HNSCC. The combination of xevinapant, radical radiotherapy, and 3-weekly cisplatin (100 mg/m$^2$) was investigated in a phase I/II study in patients with locally advanced HNSCC [80]. Phase I involved 14 patients in whom three xevinapant dose levels were tested (100, 200, and 300 mg/day) for 14 days in 3-weekly cycles; the maximum tolerable dose (MTD) was determined to be 200 mg/day and dose-limiting toxicities included transaminitis, febrile neutropoenia, and renal tubular necrosis [80]. Of note, grade 3–4 mucositis and dysphagia occurred more frequently with earlier onset in patients receiving higher xevinapant dose levels, leading the authors to suggest a potential dose-dependent radio-sensitisation effect [80]. Across all dose levels, the objective response rate was 85% and the complete response rate was 69% [80]. Given its promising preliminary efficacy, xevinapant 200 mg/day was subsequently tested in the randomized, double-blinded, phase II part of the study. Ninety-six patients with locally advanced HNSCC were randomly assigned to receive xevinapant or placebo

in combination with cisplatin-based chemoradiation [81]. The locoregional control at 18 months post-treatment, which was the primary endpoint, was superior in the xevinapant arm compared to placebo (54% vs 33%; odds ratio 2.69 (95% CI 1.13–6.42), p = 0.026) [81]. Grade ≥ 3 adverse event rates were similar between the comparator arms and no deaths due to adverse events occurred in the xevinapant group [81]. After extended follow-up, the xevinapant arm continued to demonstrate superior efficacy; the risk of all-cause mortality over 5 years was approximately halved (HR 0.47; 95% CI 0.27–0.84; p = 0.0101), the median OS was not reached (95% CI 40.3-not evaluable) compared to 36.1 months (95% CI 21.8–46.7) in the placebo arm, and the probability of maintained response at 3 years was 79% (95% CI 58%–90%) versus 36% (95% CI 16%–56%), respectively [82]. Collectively, these results indicate the safety and favourable clinical outcomes with the addition of xevinapant to standard of care chemoradiation in locally advanced HNSCC.

Two further phase I/II studies are currently investigating xevinapant in the locally advanced disease setting. The HyperlynX phase 1b trial (NCT06056310) is exploring the safety of combining xevinapant (200 mg/day for 14 days in a 21-day cycle) with radical radiotherapy and weekly cisplatin, rather than the standard 3-weekly regimen, in locally advanced HNSCC [83]. The RAVINA phase II study (NCT05724602) aims to determine the tolerability and efficacy of xevinapant during and after radiotherapy in older patients (≥70 years old) with locally advanced HNSCC [84].

The promising data from the xevinapant phase II study led to the launch of several phase III trials investigating the efficacy of xevinapant in HNSCC. The TrilynX trial (NCT04459715) is a randomized, double-blind, phase III study in which 700 patients with locally advanced HNSCC will be randomized (1:1) to receive xevinapant or placebo in combination with chemoradiation, followed by xevinapant maintenance or placebo [85]. The primary endpoint of the study is event-free survival, while the secondary endpoints are locoregional control, OS, progression-free survival, and safety [85]. As the first phase III study of an IAP antagonist in any solid cancer, the results of the TrilynX trial are eagerly awaited. Another double-blind, randomized phase III study, XXL_2022–01 (NCT05930938), is investigating the addition of xevinapant or placebo in combination with radical radiotherapy and cetuximab in locally advanced HNSCC patients who are ineligible for cisplatin [86].

In the adjuvant setting, 2 studies are investigating the efficacy of xevinapant with post-operative radiotherapy in high risk HNSCC [87, 88]. The XRay Vision trial (NCT05386550) is a randomised, double-blind, phase III study in cisplatin-ineligible patients with resected, high-risk HNSCC [87]. Around 700 patients will be randomised (1:1) to receive 6 cycles of either xevinapant (200 mg/day for 14 days in a 21-day cycle) or placebo in combination with adjuvant radiotherapy for the first 3 cycles [87]. The primary endpoint of the study is disease-free survival, and the secondary endpoints are OS, quality of life, and safety [87]. A phase II study (NCT06145412) is investigating the benefit of combining xevinapant (200 mg/m$^2$ on days 1–14 every 21 days) with post-operative radiotherapy and weekly cisplatin (40 mg/m$^2$) in resected high-risk HNSCC, with the primary outcome being 12-month disease-free survival [88].

Furthermore, tolinapant is being investigated in an early-phase, open-label study (NCT05245682) with regards to its safety and efficacy in combination with definitive or adjuvant radiotherapy in cisplatin-ineligible patients with locally advanced HNSCC [89]. Radiation treatment is delivered using either intensity-modulated radiation therapy (IMRT) or proton beam therapy, and tolinapant is administered at a dose of 180 mg/day for 7 days during weeks 1, 3, 5, and 7 of radical radiotherapy (weeks 1, 3, and 5 with adjuvant radiotherapy) [89]. Preliminary results from 5 patients suggest that tolinapant appears to be safe and well tolerated at 180 mg/day with radiation treatment, however, mature data are awaited [89].

## *Future Directions*

The observation that SMAC mimetics exert a plethora of immunomodulatory effects provides an attractive rationale to support the investigation of combining IAP antagonists with immune checkpoint agents [79]. Indeed, preliminary results from the ASTEROID phase 1 trial in 22 patients with solid tumours, including HNSCC, showed that the combination of tolinapant and pembrolizumab is safe and well-tolerated with durable anti-tumour response [90]. The overall response rate was 40%, including partial response in a patient with HNSCC refractory to immune checkpoint monotherapy [90]. Upon interrogation of paired blood samples from responders, a significant increase in cytotoxic effector T cells ($p = 0.0176$) was noted providing the first clinical proof-of-concept for the induction of immunogenic cell death by an IAP antagonist resulting in enhanced adaptive immune response when combined with immunotherapy [90]. These compelling findings warrant larger studies to explore the efficacy of combining SMAC mimetics with immune checkpoint agents in HNSCC, especially in the locally advanced disease setting.

Given the molecular heterogeneity of HNSCC and its impact on clinical efficacy with the available treatment modalities and potential future agents, including SMAC mimetics, an in-depth understanding of the genomic and microenvironmental context is warranted. This is of particular importance with the advancements in IAP antagonists' development and promising results with SMAC mimetics to guide personalised treatment decisions [91]. Finally, while the focus here has been apoptosis and its therapeutic targeting, there are numerous other programmed cell death types, the targeting of which might prove beneficial in the coming years.

The intrinsic pathway is activated by cellular stress and DNA damage leading to activation of BH3-only BCL-2 family proteins, subsequent mitochondrial outer membrane permeabilization (MOMP) and release of pro-apoptotic proteins including cytochrome c and SMAC. Cytochrome c triggers oligomerization of the apoptotic protease activating factor 1 (Apaf-1) leading to apoptosome formation and exposure of the caspase activation and recruitment domains (CARDs) which bind to pro-caspase-9. This leads to activation of caspase 9 which in turn activates caspases 3 and 7, the executioners of apoptosis. The extrinsic pathway is initiated by the binding of the death ligand FasL with its receptor FasR leading to the binding of the

adapter protein FADD, or the binding of the death ligand TNFα with its receptor TNFR leading to the binding of the adapter protein TRADD with subsequent recruitment of FADD, RIP, and TRAF2 and ubiquitination. FADD then associates with pro-caspase-8 leading to the formation of the death-inducing signalling complex (DISC) with autocatalytic activation of pro-caspases-8 and -10. Once activated, they either activate caspases 3 and 7 executing apoptosis, or activate BID into tBID with convergence onto the intrinsic apoptotic pathway.

TNFα: tumour necrosis factor alpha; TNFR: tumour necrosis factor receptor; TRADD: tumour necrosis factor receptor-associated death domain; FasL: Fas ligand; FasR: Fas receptor; FADD: Fas-associating protein with death domain; TRAF2: tumour necrosis factor receptor-associated factor 2; RIP: receptor interacting protein; c-IAPs: cellular inhibitor of apoptosis proteins; Ub: ubiquitin; IAP: inhibitor of apoptosis; BH3-only proteins: Bcl-2 homology domain 3-only proteins; tBID: truncated BH3 interacting-domain death agonist; BCL-2: B-cell leukaemia/lymphoma 2 protein; BCL-XL: B-cell lymphoma-extra large; MCL1: myeloid cell leukaemia 1; BAX: Bcl-2 associated X-protein; BAK: Bcl-2 homologous antagonist killer; MOMP: mitochondrial outer membrane permeabilization; SMAC: second mitochondria-derived activator of caspases; APAF1: apoptotic protease activating factor 1.

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# Chapter 7
# Precision Medicine Beyond Genomics

**Tessa Bray and Enrique Sanz-Garcia**

## Introduction

Precision oncology in cancer aims to improve the clinical outcomes for patients by developing individualized targeted therapy for increased efficiency and improved patient stratification [1]. The main goal of precision oncology is to move the cancer treatment paradigm from "one size fits all" to a more personalized and individualized approach. For the past two decades, the field has been evolving to include a multitude of methodologies that have resulted in an increasingly detailed understanding of the molecular features and changes that drive carcinogenesis in a wide variety of cancer types. Most of the current precision medicine-based therapies target specific protein alterations or genomic aberrations. However, as will be described further, this has limited applicability in cancer patients. Newer technologies analyzing gene expression, epigenetics, and tumour and immune cell functions are increasingly used in translational medicine and will undoubtedly help to broaden the applicability of precision medicine in more patients and tumour types (Fig. 7.1). This chapter will summarize the current landscape of precision oncology in how it relates to the treatment and understanding of head and neck squamous cell carcinoma (HNSCC). We will focus mainly on the latest developments beyond usual next-generation sequencing (NGS), including whole genome and transcriptome analysis. We will also describe some of the newest technologies that will play a role in the development of precision oncology in the next years. Finally, we will discuss

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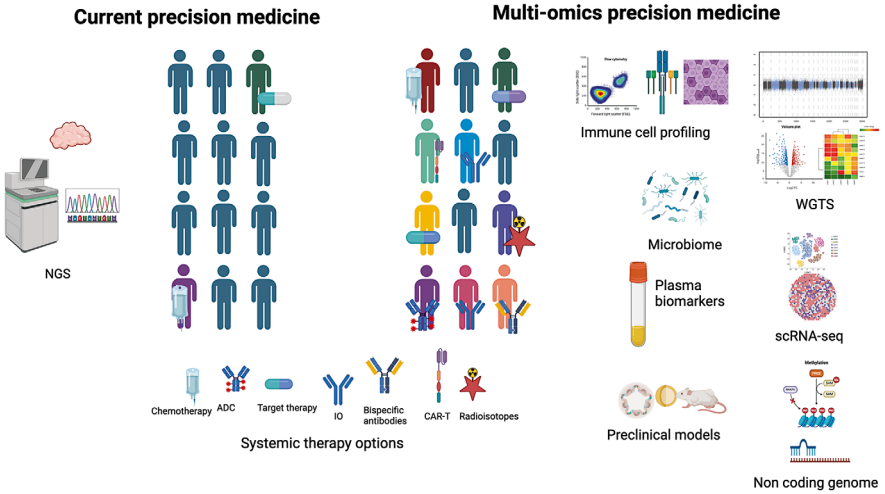


**Fig. 7.1** Precision oncology: from current paradigm based on next-generation sequencing to multi-omics approach. The multi-omics approach could broaden the applicability of precision to more patients. *ADC* antibody drug conjugates, *IO* immune-oncology, *NGS* Next generation sequencing, *scRNA-seq* single-cell RNA-sequencing and spatial transcriptomics, *WGTS* Whole genome and transcriptome sequencing. Created with BioRender.com

the current limitations of precision medicine and the hurdles to overcome for its implementation in the management of patients with cancer.

## NGS Analysis for Precision Oncology in Cancer and HNSCC

Research in the field of precision oncology has predominantly involved NGS of limited genes and subsequent matching of genomic alterations to targeted therapy options. This method was popularized because tumours often contain positively-selected genomic mutations that confer growth advantage, making many of these genes promising therapeutic targets across different cancer types [2]. NGS has had significant success at identifying therapeutic targets and predictive biomarkers in some tumour types, such as non-small cell lung cancer (NSCLC) and cholangiocarcinoma. For example, in NSCLC, there has been significant success with NGS-matched therapy with epidermal growth factor receptor (*EGFR)* mutation, anaplastic lymphoma kinase (*ALK)* translocation, and more recently Kirsten rat sarcoma virus (*KRAS)* mutations, among others [3–8]. In cholangiocarcinoma, fibroblast growth factor receptor 2 (*FGFR2)* fusions and isocitrate dehydrogenase-1 (*IDH1)* mutations have been successfully targeted with different drugs [9, 10]. Moreover, some genomic alterations such as v-raf murine sarcoma viral oncogene homolog B1 (*BRAF)* mutations, human epidermal growth factor receptor 2 (*HER2)* amplifications, neurotrophic tyrosine receptor kinase (*NTRK)* and rearranged during

transfection (*RET)* fusions seem to be targetable in different tumour types [11]. Nevertheless, these alterations are not frequent in HNSCC. The only genomic alteration that has shown some promising but not confirmed results with target therapy is Harvey rat sarcoma virus (*HRAS)* mutation. A phase II study showed a 55% objective response rate (ORR) to tipifarnib, a drug that disrupts *HRAS* function, in patients with HNSCC and high *HRAS* variant allele frequency (VAF) (>20%) [12]. However, a high *HRAS* VAF in HNSCC is not frequent (<4% of all HNSCC). In contrast, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA)* mutations are observed more frequently (13%) [13], but some of the published trials targeting that alteration in HNSCC have failed [14–16].

In general, trials evaluating precision medicine using NGS in solid tumours have shown inconsistent findings. In clinical studies using NGS to match patients to therapies based on alterations in their tumours, there is often a large discrepancy between the number of actionable alterations and the percentage of patients that receive a matched therapy. Further, potential benefit from a matched therapy is not guaranteed, as responses vary widely depending on the trial, ranging from no response to excellent clinical benefit. Some pan-cancer trials demonstrating these results have been summarized in Table 7.1. It is important to note that many of these trials include no or very low number of patients with HNSCC, further highlighting the relative lack of proven target therapies in this tumour type.

Based on the variability in responses and outcomes seen in NGS-based studies and the limited frequency of aberrations, not all the tumour types seem to benefit from this approach. However, in the era of personalized medicine, with more clinical trials available, with the decreases in cost of NGS and the advent of new drugs with high response rates for rare mutations (i.e larotrectinib), patients with advanced HNSCC should be considered to get their tumors profiled at least for the main targetable mutations. Regardless, precision medicine needs to move beyond NGS panel. The main advances in this field will be described in the next sections.

## Whole Genome Sequencing and Transcriptomics in Precision Medicine

Whole-genome sequencing (WGS) and ribonucleic acid (RNA) sequencing through bulk transcriptomics have been identified as the main new approaches in precision oncology. The analysis of all the alterations in the genome through WGS allows the detection of not only point mutations but also copy number changes and rearrangements across the entire genome, in contrast to select gene panels. Moreover, it can provide an accurate estimation of tumour mutational burden (TMB), that is the number of somatic mutations per megabase of the genome. TMB is one of the few validated biomarkers of response to immuno-oncology (IO) drugs [36, 37]. Similarly, the analysis of the gene expression (RNA-sequencing or transcriptomic) has also shed some light on some tumours without targetable alterations as a potential approach for precision medicine. Some examples are summarized below.

**Table 7.1** Largest multicentre pan-cancer clinical trials applying NGS and using basket studies in precision medicine

| Trial | Overall actionable and match | Overall benefit (any) | Specific genes studied | Tumour type | Treatment | ORR |
|---|---|---|---|---|---|---|
| NCI-MATCH [17] | 38%<br>18% | | *NRAS* mut [18] | Pan-cancer | Binimetinib | 2% |
| | | | *AKT1 E17K* mut [19] | Pan-cancer | Capivasertib | 29% |
| | | | *HER2* amp [20] | Pan-cancer | TDM-1 | 6% |
| | | | *BRAF V600E* mut [21] | Pan-cancer | Dab+Tram | 38% |
| TAPUR | | | *ERBB2* amp/mut [22] | Colorectal | Tras+Pertu | 25% |
| | | | *ERBB2* amp/mut [23] | Endometrium | Tras+Pertu | 7% |
| | | | *FLT3* amp [24] | Colorectal | Sunitinib | 0% |
| | | | *CDKN2A* mut [25] | NSCLC | Palbociclib | 0% |
| | | | *BRCA1/2* mut [26] | Prostate | Olaparib | 58% |
| | | | *RAS/RAF* wt [27] | Pan-cancer | Cetuximab | 0% |
| DRUP [28] | 66%<br>46% | ORR: 15%<br>OCB: 34% | *BRCA1/2* mut [29] | Pan-cancer | Olaparib | 29% |
| | | | *HER2* amp [30] | Colorectal | Tras+Pertu | 29% |
| | | | Multiple genes [31] | Pan-cancer (rare cancers) | Targeted therapy or IO | ORR: N/A<br>OCB: 33% |
| | | | *HER2* exon 20 mut [32] | NSCLC | Tras+Pertu | 8% |
| My Pathway [33] | | ORR: 23% | *HER2* mut [34] | Pan-cancer | Tras+Pertu | 6% |
| | | | *HER2* amp [35] | Colorectal | Tras+Pertu | 32% |
| | | | *BRAF V600E* mut [33] | Pan-cancer | Vemurafenib | 23% |

*amp* amplification, *Dab + Tram* dabrafenib + trametinib, *IO* immune oncology, *mut* mutation, *N/A* not applicable, *NSCLC* non-small cell lung cancer, *OCB* overall clinical benefit (partial or complete response or stable disease > 6 months), *ORR* objective response rate; *TDM*-1 ado-trastuzumab emantsine, *Tras + Pertu* trastuzumab and pertuzumab, *wt* wild type

One of the first studies to explore the application of precision oncology beyond targeted NGS panels is the WINTHER study [38]. This international multicentre clinical trial aimed to assign patients to a specific treatment based on genomic alterations using NGS. However, if a target/match was not found, analysis of RNA expression was used to assign a therapy. Of the 303 patients screened for the study, only 107 (35%) were eligible to receive matched treatment of which 69 were matched to targeted treatment via NGS, and 38 through the transcriptomic analysis (22.7 and 12.5% of patients originally screened, respectively). A total of 21 HNSSC cases were included. The primary endpoint of the study was centered on the ratio of PFS2/PFS1, where PFS2 represents progression-free survival (PFS) during the trial and PFS1 represents PFS on the most recent therapy before enrollment for each patient. This primary endpoint was not met, given that less than 40% of patients who were treated with a matched targeted therapy had PFS 1.5 times greater on the trial (PFS2) than PFS on their most recent previous treatment (PFS1). However, interesting results were observed when comparing a DNA-guided approach versus an RNA-guided approach. First, a higher rate of clinical benefit (stable disease for over 6 months or radiological response) was observed in therapies matched using RNA sequencing (12/38, 31.6%) compared to therapies matched through DNA alterations (16/69, 23.2%). Second, the PFS2/PFS1 ratio > 1.5 was slightly higher in the patients whose treatment was selected based on RNA-sequencing (26.3% versus 20.3% in NGS-based therapies). Further, the application of transcriptomics for treatment matching increased the number of patients treated on the trial (from 23% to 35%), even though matching according to transcriptomic analysis was done on an exploratory basis. RNA-based therapy selection also had at least a similar impact (if not slightly better) in outcomes such as clinical benefit and PFS compared to NGS approaches. Therefore, this large study lays the groundwork for the idea that RNA sequencing could be further explored as a useful tool for matching patients to targeted therapies.

The Personalized OncoGenomics (POG) program is another important study that explored the application of whole analysis of the genome and transcriptome in precision oncology (Whole Genome Transcriptomics Analysis or WGTA) [39, 40]. This study was larger than WINTHER in terms of enrolled patients (N = 722), with a variety of cancer types including HNSCC. Nearly 80% of the patients had WGTA results to guide potential treatment selection. Overall, 66% of the initial study population were identified to have clinically actionable alterations in the WGTA according to a molecular tumour board, but only 29% were treated with a therapy informed by WGTA. Most patients did not receive matched therapy despite actionable alterations due to death/poor performance status or lack of access to the proposed treatment. However, among the group of patients that received WGTA-informed therapy, 46% demonstrated clinical benefit. Despite the low clinical benefit in the overall population (13% of all the patients who were screened), this metric is slightly higher than in the WINTHER study. Indeed, the combined use of more than one output from the WGTA (mutation, copy number, structural variants, genome signature, or RNA expression) directed treatment in the majority of the patients. Notably, RNA expression was the method that contributed most commonly to the WGTA-informed

therapy (up to 67% of all the cases that were matched to a therapy), but the clinical benefit did not seem to differ among genome or transcriptome-guided approaches. The therapies that were more frequently matched were tyrosine kinase inhibitors and inhibitors of the homologous recombination deficiency pathway, as well as immunotherapy based on programmed death ligand 1 (PD-L1) expression and other immune-related signatures. Overall, the POG study shows that WGTA has a potential role in precision medicine, although its cost and low applicability remain an issue to solve.

## Gene Expression Profiling in HNSCC

As previously mentioned, WGTA has not been analyzed extensively in clinical trials with patients with HNSCC. However, different studies have suggested the potential use of RNA analysis to guide therapeutic decisions. Some examples such as hypoxia or anti-EGFR gene expression signatures or signatures that predict response to IO are summarized in this section.

**Hypoxia Gene Expression Signatures**
A hypoxic tumour microenvironment (TME) has been recognized as a therapeutic challenge in HNSCC, as these tumours exhibit a reduced response to radiotherapy. As such, it may be relevant to identify tumours with high hypoxia to improve outcomes. A proposed potential biomarker for hypoxia was suggested using RNA sequencing. A 15-gene expression signature was generated to classify patients as having either a high or low level of hypoxia [41]. This signature was validated by the same working group in the prospective DAHANCA-5 study, in which patients with HNSCC were treated with nimorazole, a hypoxic radiosensitizer, and radiotherapy [42]. The study found that patients with high expression of the hypoxia signature did significantly benefit from nimorazole with improved loco-regional tumour control (LRC) and disease-specific survival. In contrast, those patients where a low expression of the signature was observed did not have significant benefit from the addition of the hypoxia inhibitor [42]. This signature (in addition to another signature involving a total of 30 genes) was able to successfully distinguish HNSCC patients with good and poor prognosis, indicating the possibility for hypoxia gene expression signatures to be applied as a prognostic tool in combination with their potential predictive value [43]. This hypoxia signature was intended to be validated in a multicentre large study (EORTC1219/DAHANCA 29) where patients were randomized to cisplatin-based concurrent chemoradiotherapy (CCRT) plus nimorazole versus CCRT plus placebo. Unfortunately, the recruitment was closed early and there were no differences in LRC neither in the entire population nor in the patients with high expression of this signature [44].

**Gene Expression Profiling to Predict Anti-EGFR Therapy Outcomes**
RNA-based signatures have similarly been proposed to predict benefit from anti-EGFR therapy such as cetuximab or panitumumab. Before the introduction of IO,

cetuximab was frequently used in recurrent/metastatic HNSCC patients in combination with platinum-based chemotherapy [45]. However, despite proven clinical benefit, many patients did not respond in the long term to this combination. A RNA signature was proposed as a potential tool to select those with and without benefit to anti-EGFR [46]. Briefly, RNA-sequencing was performed in patients with long and short PFS and found that a total of 7 genes were expressed differently among both groups. A signature characterised by a basal subtype and hypoxia was associated with prolonged responses to cetuximab. Moreover, the same signature was associated with afatinib (another EGFR inhibitor) in preclinical models. This signature predicted metabolic (using positron emission tomography, PET/CT) but not radiological response in a window of opportunity clinical trial in patients treated with afatinib before surgery [47]. Similarly, in the metastatic setting, this signature seemed to predict PFS in patients treated with panitumumab as a single agent in the PANI-001 study [48]. Nevertheless, this signature was not further explored.

**Transcriptomics to Predict Response to IO**

The application of transcriptomics in precision oncology is increasingly focused on the potential to predict response to IO in many cancer types. This includes HNSCC. Pembrolizumab has shown benefit in the first-line setting alone or in combination with chemotherapy or as monotherapy in second line. Similarly, nivolumab monotherapy has shown benefit after first-line palliative treatment or in platinum-refractory patients [49, 50]. To date, the only validated biomarker of response to programmed death-1 (PD-1) antibodies in this setting is PD-L1 expression by immunohistochemistry for pembrolizumab [51]. TMB, as mentioned above, has been also suggested as a potential biomarker to predict ICB benefit. Despite these advancements and the improved survival seen in some patients, most patients treated with immune checkpoint blockade (ICB) do not derive clinical benefit from the drugs. There is a clear need for more predictive biomarkers to improve patient selection, which has prompted the research and development of a multitude of transcriptomics signatures. Some examples are highlighted below.

One of the first proposed signatures to predict outcomes to ICB (anti-PD-1 in particular) was a T-cell inflamed gene expression signature [52]. This signature contained a total of 18 differentiated regulated genes involved in interferon-gamma response to antigen presentation, chemokine expression, cytotoxic activity, and adaptive immune resistance, as well as PD-L1 expression. This signature was validated in conjunction with TMB in four clinical trials with pembrolizumab in 22 different tumor types [53]. A significant positive correlation between the expression of this signature and response to pembrolizumab across different tumour types including HNSCC was observed, supporting the notion that a T-cell inflamed TME could be associated with a potential benefit from ICB. A recent joint analysis of different trials applied a total of eleven signatures including the T-cell inflamed one. Surprisingly, this signature was not the only one associated with outcomes of pembrolizumab. Others, such as a signature for angiogenesis, myeloid-derived suppressor cells, and stroma, were negatively associated with response, showing that just one signature may not be able to accurately predict outcomes [54].

Another more recent gene expression signature was created based on a cohort of patients who had received IO (VIGex). This was generated using Nanostring technology and a total of 12 genes associated with the TME were selected to predict the outcomes of IO [55]. VIGex scores were created according to the inflammatory status of the TME, and tumours were stratified in hot, intermediate-cold, and cold. It was found that the hot phenotype was associated with greater clinical benefit and longer PFS and overall survival (OS) compared to the cold phenotype in patients treated with anti-PD-1/PD-L1 agents in early-phase clinical trials. Interestingly, this signature was evaluated in a dataset of HNSCC patients treated with ICB, and these categories were also associated with PFS and OS. The findings attributed to the VIGex signature were further validated in the same work through a meta-analysis of publicly accessible gene expression datasets of patients treated with IO, suggesting that the predictive power of the signature is consistent across datasets and tumour types. Interestingly, VIGex and TMB were not associated, indicating the potential for VIGex to be explored further as an independent predictive biomarker for ICB in clinical trials.

Similarly, another gene-expression-based score (HOT score) has been suggested to identify immunologically active HNSCC tumours [56]. The HOT score was created using gene expression profiles and clinical data from 520 HNSCC tumours of The Cancer Genome Atlas database, and it was validated through its association with immune markers of PD-L1 expression, interferon-gamma signature, and tumour infiltrating lymphocytes (TILs). The score was then tested clinically, by classifying 102 HNSCC patients treated with PD-1/PD-L1 inhibitors as either a 'hot' or 'cold' phenotype. Those with the 'hot' phenotype had comparatively better OS and PFS. The HOT score was also found to be associated with higher ORR and duration of response to ICB. These findings are consistent with the results seen with the VIGex signature, supporting the potential clinical utility of signatures that identify immunologically inflamed tumours.

Given the number of immune-related transcriptomics signatures that have been developed in the past few years, a meta-analysis has been performed to evaluate the most promising signatures for informing sensitivity and resistance at a pan-cancer level and generate a joint signature that could overlap all the previous signatures [57]. Out of 37 identified signatures, 22 were associated with at least one clinical outcome, and 10 were associated with all three (OS, PFS, and ORR). To gain deeper insight into the mechanism behind the response to ICB, a meta-analysis was performed including 12,329 genes from all the transcriptomics studies. The top 100 genes associated with a response to ICB were identified and grouped into the PredictIO signature (77 associated with ICB response and 23 associated with resistance). PredictIO score is defined as the ratio calculated between the expression of the response and resistance genes using the gene Set Variation Analysis (GSVA), a method that estimates variation of pathway activity in an unsupervised manner. This score was tested in different tumour types including HNSCC. PredictIO was significantly associated with ICB response across seven independent patient cohorts, better than each of the previous signatures. Additionally, a high PredictIO score was

significantly correlated with longer PFS to antiPD-1 therapy in a dataset of pan-cancer patients treated with pembrolizumab and another dataset of patients with melanoma treated with antiPD-1 +/− anti-CTLA-4 therapy.

## Single-Cell RNA-Sequencing and Spatial Transcriptomics

All the abovementioned signatures involved bulk transcriptomics which is a form of RNA-sequencing that produces an average expression level of each gene across all cells in the tissue sample. However, the gene expression is different between the cells that are involved in a tumour (i.e. tumour cells, immune cells, fibroblast, etc.). New technologies have made the transcriptomic analysis possible at a resolution of a single cell (single cell RNA-sequencing) [58]. This approach can characterise the transcriptome of an individual cell. One of the main steps of this technique involves the isolation of cells leading to a loss of the spatial distribution of these cells [59]. Spatial transcriptomics has emerged to overcome this limitation, helping to localise the RNA transcripts to a precise spatial location. There are at least two different approaches for these methods: high-plex RNA imaging [60] and spatial barcoding [61].

Single-cell and spatial transcriptomics are currently developed in translational research with the goal of understanding better the role of different genes and pathways in the development or progression of cancer. Moreover, there is a growing interest in developing these techniques to discover new biomarkers of prognosis or response to therapies [62]. Recent studies have shown potential applications of single-cell RNA-seq and spatial transcriptomics in head and neck cancer. Single-cell RNA-sequencing studies have suggested a subset of cells associated with progression and metastases and may be the target for potential therapies [63, 64]. Spatial transcriptomics has also been suggested as a potential approach for precision oncology. For instance, a study was able to discriminate two main areas on HNSCC tumours: the tumour core (which is characterised by the expression of genes related to keratinization) and the leading edge (which showed mainly expression of genes related to collagen) [65]. Interestingly, the leading edge is enriched with immune cells (mainly cytotoxic CD8+ T-cell) while macrophages were more frequent in the tumor core. One of the main findings of this elegant work was an in-silico analysis showing that drugs with a greater benefit, induced the cells from the leading edge to transition to the tumour core. Therefore, the leading edge (and not the whole tumour) may be the area to focus on for drug discovery and analysis of potential biomarkers of response to therapies. Another study has also suggested that spatial transcriptomics could help discover and rank clinically relevant alternative medications, preventing the use of treatments that may not be effective while identifying new targets [66]. If these findings are validated in larger studies, spatial transcriptomics could become a potential tool for precision medicine in solid tumours.

## Immune Cells and T-Cell Receptor (TCR)

With the advent of checkpoint inhibitors in HNSCC, especially anti-PD-1 antibodies, biomarkers of response to this therapy beyond PD-L1 are actively sought [50]. Apart from the above mentioned transcriptomic signatures, many other potential biomarkers have been evaluated. One of them is the tumor microenvironment, and more specifically the presence of specific immune cells. Different methods have been used to analyze these cells in HNSCC including the previously mentioned single-cell RNA-seq [67], multiplex immunohistochemistry, or flow cytometry [68], among others. Many cells are relevant in the TME of HNSCC and could be associated with the sensitivity or resistance not only to anti-PD-1 therapy but also to chemotherapy or radiation [69]. Among them, TILs have been widely studied. For instance, TILs with decreased effector function (measured as high expression of immune checkpoints, co-expression of CD39/CD103 or upregulation of activation markers) have been associated with prolonged responses [70]. Similarly, high CD8+ T cell infiltration has been correlated with better response to anti-PD-1 therapy in HNSCC [71]. Recently, B-cells in the stroma but not in other immune cell populations have been associated with prolonged PFS and OS to anti-PD-1 therapy [72]. However, there is a lack of prospective validation of these immune cell types as predictive biomarkers of response, especially in clinical trials.

Similarly, significant research is ongoing regarding the analysis of the characteristics of the T-cell receptor (TCR), which is the effector of the immune response induced by the checkpoint inhibitors [73]. Lately, the analysis of the TCR repertoire (the characteristics of the TCR chains in tumours or blood) has been possible due to the development of new technologies such as TCR sequencing [74]. Two concepts are critical to understanding TCR in response to IO: clonality and diversity [75]. Clonality is the amount of TCR sequences that are targeting a specific antigen. Diversity is the amount of different TCRs against different antigens or the number of unique TCR sequences. Both characteristics, despite being opposite, need to be well balanced for the development of an immune response. Diversity and clonality of TCRs have been associated with the response to anti-PD-1 in solid tumours. For instance, high clonality was predictive of prolonged survival in patients treated with melanoma who were treated with anti-PD1. Similarly, high diversity was prognostic in different solid tumours [76]. TCR diversity and clonality have also been associated with outcomes in HNSCC. For example, in a clinical trial involving nivolumab and cetuximab, the likelihood of response was higher in those patients with higher clonality and diversity in plasma, especially in HPV-negative tumours [77]. A lower TCR diversity has been reported in HNSCC compared to other tumor types. Similarly, lower TCR diversity has been observed in acquired resistance to IO [78]. Furthermore, the dynamics of the TCR diversity and functional clonal annotation are emerging as a potential biomarker of response to anti-PD-1 [79].

Despite immune cell profiling and TCR-sequencing being promising for the discovery and validation of biomarkers of response to therapies in HNSCC, the complexity and high cost of some of these methods precludes currently the application in the daily practice as a tool of precision medicine.

## Non-coding Genome

One of the main areas of development of precision medicine in the near future is the analysis of the non-coding genome. Over many years, this part of the genome was called the "junk genome" and was presumed not to have any role in cancer or in response to therapies. However, this does not seem to be the case anymore. A large study including more than 2000 different genomes showed that mutations and structural variants in this part of the genome, although being less frequent than in the coding genome, can still play a role in driving cancer and predicting response to therapy [80]. The increasing use of WGS in clinical and translational research is helping to better understand this part of the genome. Non-coding genome also includes other areas of the DNA, which are important in the transcription and regulation of the coding genome. For instance, areas such as enhancers or promoters that induce gene expression, or others such as insulators that prevent transcription [81]. Moreover, some areas of the non-coding genome are responsible for the transcription of non-coding RNA. All these areas are important for transcription factor binding, chromatin openness (the characteristic of the chromatin to remain open or closed for binding of the different DNA regulators), histone modification, and change in the three-dimensional (3D) structure of DNA [82]. Apart from WGS that is already available in many research centers, other techniques such as assay for transposase-accessible chromatin using sequencing (ATAC-seq) or chromatin immunoprecipitation sequencing (CHIP-seq) are making possible to better decipher the non-coding genome and look for potential targets in precision oncology [83, 84]. For instance, in preclinical models of HNSCC, ATAC-seq has shown potential changes in the non-coding genome associated with some activated pathways in tumour cells treated with cetuximab [85]. One of the limitations at this point of this approach is the lack of understanding of many events occurring in the non-coding genome and how these events are related to cancer development or therapy response. Recently, a pan-cancer atlas has been constructed to elucidate some of these findings, including in HNSCC [86]. However, the application of these chromatin analyses are still far from being used as a standard tool for precision oncology due to the lack of technical and bioinformatic expertise as well as the need for fresh biopsies to perform these analyses. Nevertheless, some promising data is emerging in formalin-fixed paraffin-embedded samples that could open a new dimension in the use of these methods to discover biomarkers of response or potential targets [87]. From a therapeutic perspective, there are many drugs targeting the epigenome (which is usually regulated by the non-coding genome). Some of them are well known and used in haematological malignancies such as DNA methyltransferase inhibitors (azacitidine and decitabine) and histone deacetylation inhibitors (vorinostat, romidepsin, panobinostat, and belinostat, among others). Some of these drugs have been tested in HNSCC with modest benefit (i.e. vorinostat) [88]. Others such as bromodomain and extra-terminal motif (BET) inhibitors have been more recently developed and had some efficacy in solid tumours including HNSCC with a toxicity profile that needs to be better defined and studied in larger studies [89–91].

## Patient-Derived Cancer Models

One area of basic and translational research that has the potential to become a precision medicine tool for patients with cancer is the use of patient-derived models such as patient-derived xenografts (PDX), organoids or spheroids, among others. These models could resemble the original tumour and be used to test potential therapies in vivo, offering a unique and personalized approach for each patient [92]. PDX are established from tumour cells into immune-deficient animal hosts with a variable success rate of establishment [93]. In HNSCC, PDX have been reported to be useful to test personalized medicine [94]. Recently, PDX have been successfully established from patients with recurrent/metastatic HNSCC and in a few cases, their later lines of treatment were guided by these PDX. Of note, a good response to a therapy suggested by the PDX was observed in one patient [95]. Organoids (in vitro 3D structures that are established from patient tissue) from HNSCC have also been recently reported as potential tools to predict treatment response to cetuximab, radiation or target therapy [96]. Spheroids (3D micro-aggregates of cancer cells) have also been tested to predict response to radiation in HNSCC [97]. Despite these promising results, these patient-derived cancer models have important limitations, such as the low success rate in establishment, the time required to develop them and make them useful for testing therapeutic options, and significant cost.

## Microbiome

The microbiota (the different microbiome species that are usually part of the human body) are relevant to regulating the immune function and protecting against external pathogens. However, changes in the microbiota (known as dysbiosis) have been implicated in the pathogenesis of cancer, including HNSCC, by inducing apoptosis, inflammatory reactions, or damaging DNA [98, 99]. Moreover, chemoradiation in HNSCC has been associated with a shift in the oral cavity microbiota showing an increase in the relative abundance of gut-associated microbiota [100]. This change is not observed in the intestinal microbiota, meaning that systemic therapy does not produce significant changes and is mainly driven by radiation. In patients treated with IO, different baseline microbiota was observed in responders and non-responders, especially in melanoma [101, 102]. Therefore, personalized strategies have been suggested to switch the microbiome to a composition more favourable for treatment response. Among these strategies, fecal microbiota transplantation (enriched from species that are associated with better outcomes, such as *Ruminococcus, Faecalibacterium* and *Eubacterium,* among others) has been successfully performed in patients with solid tumours treated with anti-PD-1 (melanoma, hepatocarcinoma, and oesophageal cancer) [103, 104], but not yet in HNSCC. As a less complex and potentially larger-scale alternative, microbial

consortia (multi-species mixtures of cultivated microbiome) have also been tested with IO in solid tumours, including head and neck cancer [105]. However, trials evaluating these personalized microbiome therapies, are still in very early phase and have only shown safety and potential engraftment of some of the bacteria in the patient's intestine. If this approach will potentially impact in the outcomes of IO remains to be seen.

## Plasma Biomarkers as a Tool for Precision Medicine

Most of the previously mentioned analyses for precision medicine have been performed in tumour tissue (except the TCR sequencing and microbiome). However, the reality is that there is significant intratumoural heterogeneity within each tumour but also between different sites of metastases. Therefore, many of the precision medicine tools developed so far may be limited in capturing this heterogeneity [106]. Moreover, the need for fresh biopsies, which may not be feasible in all patients, is another limitation of some of these methods (spatial transcriptomics, single cell sequencing, ATAC-seq). In that sense, a non-invasive analysis of tumour-derived components in fluids (also known as liquid biopsy) is emerging and being progressively established as a potential alternative to solid tumour biopsy.

ctDNA is by far the most known tumour-derived component in plasma. ctDNA has been successfully applied for tumour genotyping and treatment monitoring and is currently being explored in molecular/minimal residual disease (MRD) detection after curative treatment, and there is hope that it could be used for early cancer detection. In HNSCC, the use of ctDNA has been explored in clinical trials to evaluate the efficacy of some targeted therapies [15]. Similar to tumour sequencing, genomic profiling in ctDNA in HNSCC is limited [107]. On the other hand, the use of ctDNA to monitor response to systemic therapy seems to be more promising using a mutation-based ctDNA approach [108, 109] and most recently, a methylated-based approach (which may be more applicable, as it does not rely in available tumor tissue for sequencing and/or presence of mutations) [110]. In comparison with other tumours such as colon cancer, molecular residual disease detection in HNSCC is still under early stages of investigation. Different approaches have been suggested from assays that track specific plasma mutations based on the presence of specific mutations in the surgical specimen [111] to tumour-naïve fixed gene panel ctDNA [112]. However, the best moment to detect MRD and the best methodology are still unclear and need some refinement before implementation [113]. Metabolites have also been suggested to be measured in plasma and could be a potential tool for precision oncology. Some metabolites are increased in the blood, urine, and saliva of patients with HNSCC compared to healthy controls [114]. Other metabolites have been associated with response to chemoradiation [115] or IO [116].

## Limitations to the Implementation of Precision Medicine in Clinical Practice

Despite all these advances in technologies and potential tools for precision medicine, there is still a long way to get these methods implemented in clinical practice, including NGS approaches, which are currently available for other tumours such as lung cancer [117]. First, there is still not much known regarding the potential utility and application of all these "multi-omics". In that sense, national and international consortiums, such as the GENIE consortium led by the American Association of Cancer Research [118], are working to leverage all the information and make it publicly available to all the scientists. Second, all these approaches need to be validated in multicentre, ideally randomized, studies that could show if those technologies are useful to improve the outcome of cancer patients compared to standard-of-care approaches. So far, despite individual cases of improvement in outcomes with this strategy, this data is not yet available. Moreover, the success of precision medicine relies partially on integrating all the results provided by all the different approaches. Artificial intelligence and machine learning are great opportunities to integrate all this information [119]. For instance, MatchMiner has successfully been used to link clinical and molecular information with potential treatments and clinical trials [120]. However, the most limiting factor that precludes the use of all these approaches in real-time and for patient benefit is the high cost [121], even with "cheaper" targeted genomic sequencing which does not necessarily translate to improved survival outcomes [122]. An attempt to simplify precision medicine tools is being led by the use of digital pathology and artificial intelligence [123]. Some are based on pathological and clinical characteristics to construct a model to predict recurrence or outcomes [124]. Others are more complex and based on pathology; they can decipher a transcriptomic profile based on a single haematoxylin/eosin slide and link to a potential response to treatment [125, 126]. These results may increase the accessibility to precision oncology to more patients and decrease the costs of all the other technologies. Once again, despite these promising results, there is still a long way to get this approach validated and implemented in clinical practice.

## Conclusion

Precision medicine in HNSCC is not as widespread as in other solid tumours. Genomic sequencing can potentially unveil alterations in a small percentage of patients that can be targetable with experimental or approved therapies, albeit with variable efficacy. The advent of WGS and transcriptomics is opening the door to expanding treatment opportunities in HNSCC, especially to select patients who will have response to anti-EGFR and IO. Nevertheless, a minority of patients have access to and/or are eligible for such complex testing. New approaches such as spatial transcriptomics, immune profiling, and non-coding genome analysis are starting

to be tested in cancer patients but with no definitive implication in treatment decisions so far. One of the next steps in precision oncology is to move beyond the tumour and consider other variables, such as microbiome and plasma tumour-derived substances to select the best treatments. The use of artificial intelligence and cost reduction will help precision medicine to move forward and change from the one-size-fits-all paradigm to a more individualized approach.

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# Part II
# Primary Disease

# Chapter 8
# Sentinel Lymph Node Biopsy in cN0 Early-Stage Oral Cavity Cancer: What Is the Evidence?


**Eddy Lam and Clare Schilling**

## Introduction

Cervical lymph node metastasis has a significant impact in the prognosis of head and neck cancers. 5-year overall survival is reduced by 50% once the regional lymph nodes are involved [1–3]. Thus, the accurate assessment of the lymph node status with timely and appropriate treatment are crucially important, especially for the apparently node negative (cN0) patient.

The sentinel lymph node (SLN) is defined theoretically as the first lymph node or nodes reached by the primary lymphatic drainage of a malignant tumor, a concept first introduced by Gould et al. [4] in 1960 to stage the neck in parotid cancers. In the case of absence of malignant cells in the SLN, it is assumed the remaining regional lymph nodes will also be free of metastasis.

Sentinel lymph node biopsy (SLNB) has been widely investigated and validated in various cancers, including endometrial cancer [5], penile cancer [6] colorectal [7], thyroid [8] and lung cancers [9]. It is universally adopted in the staging of early breast cancer and melanoma with convincing evidence to demonstrate the comparable survival outcomes and similar risks of regional recurrence between patients undergoing SLNB and those with upfront regional lymph node excision [10–12]. Furthermore, a recently published meta-analysis comprising 145,548 women with clinical node-negative early breast cancer found that no further treatment (axillary lymph node dissection or axillary radiotherapy) in patients with positive SLN did not compromise the oncology outcomes [13] and complications due to axillary

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lymph node dissection including lymphoedema, infection, paresthesia and seroma, can be avoided.

In this chapter, we will comprehensively analyze the current evidence for SLNB and alternative management of the cN0 neck in oral cancer.

## Occult Lymph Node Metastasis in Head and Neck Cancers

Squamous cell carcinoma (SCC) is the most common histological type of head and neck cancer (HNC). Depending on the primary site, head and neck squamous cell carcinoma (HNSCC) has different propensity for regional lymph node metastasis.

Cancer of the oral cavity has a relatively high risk of early lymph node metastasis with up to 30% of cN0 early (T1/T2) oral cavity cancer patients harboring occult cervical metastasis in the elective neck dissection [14–17]. Within the oral cavity, primary tumor arising from different anatomical subsites also exhibit different frequency and pattern of the regional metastasis [18–22]. Tumor close to the midline exhibited higher probability of bilateral or contralateral metastasis [20–22]. Table 8.1 shows summary of prevalence of occult metastasis in clinical node-negative early oral cavity cancer based on different anatomical subsites [18, 23–25]. It should be noted this evidence is based on very few and historical studies that differentiate occult metastatic rate based on anatomical subsite.

Due to the risk of occult cervical metastasis, proactive treatment of the neck would appear to be justified, however patients with node-negative disease are still in the majority. The additional risks of treatment such as neck and shoulder stiffness and lower lip weakness must be considered in the management decision of clinically node-negative early oral cavity cancer.

**Table 8.1** The prevalence of occult regional metastasis based on the oral cavity subsites

| | Prevalence (%) | |
|---|---|---|
| Subsites of oral cavity cT1-T2 N0 OSCC | Ipsilateral neck metastasis | Contralateral neck metastasis |
| Oral tongue | (10.2–44.6) 24.4[a] [17] | 3.8–5.5[b] [23, 24] |
| Floor of mouth | 26.3 [18] | |
| Gingiva | 38.5 [18] | |
| Buccal mucosa | 43.8 [18] | |
| Retromolar trigone | 55.6 [18] | |

[a] Mean rate of occult metastasis (95% CI: 0.205–0.248; meta-analysis comprising of 19 studies, total 1567 cT1-2 N0 oral tongue cancer)

[b] Contralateral occult metastasis is rare. No study has large number of patients for subsite analysis

## Observation of the cN0 Neck in Oral Cancer Is Not a Reliable Treatment Option

Watchful waiting with therapeutic neck dissection in the case of subsequently manifested nodal disease has been a proposed treatment option for the clinically nodal negative early oral cancer. However, several studies including randomized controlled trials have shown a higher risk of recurrent disease together with worse survival outcomes among patients with observation followed by salvage treatment compared with an upfront elective neck dissection [14, 15, 25, 26].

In 2015 D'Cruz et al. [14] published their trial of 496 T1-T2 oral cavity squamous cell carcinoma patients randomized to elective neck dissection or watchful wait (therapeutic neck dissection). Originally designed to recruit 710 patients, the interim analysis showed significant 3-year overall and disease-free survival benefit of 12.5% ($p = 0.01$) and 23.6% ($p < 0.001$) in the elective surgery group and as a result the trial was stopped early. Data showed the therapeutic surgery group presented with a more advanced nodal stage together with a higher incidence of extracapsular spread.

These results were echoed in the UK national prospective SElective Neck Dissection Study (SEND) recruiting 250 patients from 25 UK centers. The elective neck dissection arm clearly demonstrated an absolute benefit of 17.9% ($p = 0.003$) and 15.8% ($p = 0.045$) in the 5-year disease-specific survival and loco-regional recurrence compared with primary tumor resection alone [15].

Given more positive findings favoring END from multiple study cohorts and randomized trials, it has prompted subsequent meta-analyses to assess the confidence these findings. In 2020, a comprehensive systematic review involving eight randomized or matched case-control studies with a total of 2165 cT1-2 N0 oral cavity cancer patients further concluded the benefit of upfront elective neck dissection compared to observation. Significant reduction in the nodal recurrence (OR: 0.25, $p < 0.00001$), better overall survival (OR:1.95, $p < 0.0001$) and disease specific survival (OR:1.88, $p = 0.005$) were found in the meta-analysis for pooled-END group compared with the observation counterpart [27].

As a result, elective unilateral or bilateral neck dissection including the nodal region of highest risk for occult metastasis are the widely adopted gold standard in treatment of clinical N0 early oral cavity cancer globally and included in national guidelines [28–30].

## Problems Related to Elective Neck Dissection

Although END for the clinical nodal negative disease can successfully achieve a lower risk of regional recurrence compared to watchful wait, there is still a failure rate in the neck. Meta-analysis conducted by Chengini et al. [31] involving 4824

pathologically N0 oral cavity cancer patients from 21 prospective and retrospective studies revealed 13.0% isolated neck recurrence after pN0 END. Of these, six datasets comprising of 466 patients showed 2.6% recurrence in the contralateral neck meaning the majority of recurrences were in the previously dissected neck.

The randomized cohorts for the END arms from SEND [15] and D'Cruz trials [14] also demonstrated a similar finding, with regional recurrence rate of 15.1% (19/126) and 10.3% (25/243) respectively. There were several possible reasons accounting for the regional failure, including inadequate clearance of required nodal levels during the initial END, or the "skip metastasis" to the other undissected nodal basins, and potential tumor seeding during the procedure. The incidence of a truly "skip metastasis" to level IV lymph node has been estimated from 2 to 5.5%, and the oral tongue was the commonest primary site [18, 32, 33]. Level V metastasis is quite rare in clinically N0 disease, with an estimated incidence of less than 1% [18]. However, these isolated metastases outside the highest risk nodal basins can possibly be illustrated by the lymphoscintigraphy for SLNB. It represents the less common pattern of the regional metastasis due to the more individualized lymphatic drainage pattern.

Another possible reason would be under-reporting of the initial neck dissection specimen by the routine histopathology. With the routine section and H&E staining only, it is not uncommon to miss some micro-metastases and isolated tumor cells (ITC) in the dissected small lymph nodes. Serial section of each lymph node with an additional cytokeratin immunostaining can definitely increase the sensitivity to detect small occult metastasis, ranging from 15–20% [34–37]. This was further illustrated by Amit et al. [38] and Ganly et al. [39] showing 15.0 and 13.4% of pN0 END specimens respectively were upstaged following serial section analysis.

Although elective neck dissection in the primary disease-free neck is relatively safe in experienced head and neck cancer centers, it is still an invasive treatment modality not without risk. Since 70–80% clinically nodal negative oral cancer patients have pathologically proven negative disease, that means they are overtreated at the expense of some potentially avoidable surgical risks, longer hospital-stay and higher cost of treatment.

The risks of elective neck dissection include both intra-operative complications and long-term morbidities, such as infection, post-operative hematoma (1.1–1.3%) [40], chyle leak (1–2.5%) [41], shoulder dysfunction (0–94.3%) [42], and marginal mandibular nerve injury (5.5–22.5%) [42, 43].

In the adverse event analysis of the SEND randomized cohort comprising 250 patients, those with END suffered more frequently from a grade 3–4 facial or neck nerve injury (3% versus 1%, $p < 0.001$) and swallowing problem (4.8% versus 1.6%, $p = 0.03$) compared to the watchful wait arm [15].

## Minimal Morbidity Without Compromising Oncological Outcomes, Is SLNB an Alternative to END?

As both elective neck dissection and observation have their disadvantages, the emergence of SLNB seems to be a potential alternative option between these two polarized stances.

If sentinel lymph node biopsy is an optimal alternative, the survival and functional outcomes of patients staged by this method should be at least equivalent to those undergoing ELND.

### *Oncological Outcomes of SLNB Compared to END*

In a phase III prospective randomized non-inferiority multi-institutional study in Japan, Hasegawa et al. [44] randomized 271 cT1 cases with depth of invasion (DOI) more than 4 mm and cT2 oral cancer patients to undergo SLNB or END. Designed with a non-inferiority margin of 12%, the 3-year overall and disease-free survival of SLNB vs. END was 87.9% versus 86.6% ($p < 0.001$) and 78.7% versus 81.3% ($p < 0.001$), respectively, allowing a conclusion of non-inferiority in survival.

Similarly, a prospective randomized phase III multi-center study in France (Senti-MERORL) [45] involving 10 centers and randomizing 307 cT1-T2 patients demonstrated a similar 2-year recurrence free survival in both SLNB and END arms (9.3% versus 10.1% respectively, $p = 0.82$). The study was designed with a hypothesis of a 10% difference to show equivalence, and the final absolute difference of 1.1% concluded that management of clinically N0 oral cancer by SLNB was as safe as END in terms of disease survival ($p < 0.01$).

Gupta et al. [46] further demonstrated the equivalent oncological outcome between SLNB and END after systemically reviewing three randomized trials encompassing 608 patients with clinical N0 early oral or oropharyngeal SCC. The pooled hazard ratio for overall survival and relative ratio of regional recurrence were 1.18 ($p = 0.41$) and 1.11 ($p = 0.66$) respectively. However, the quality of evidence assessed by Grades of Recommendation, Assessment, Development and Evaluation (GRADE) approach [47] was low for both the oncological outcome and neck function suggesting further research is still warranted to verify the importance of these findings.

In a more recently published meta-analysis comprising of 12 studies with 10,583 early oral cancer patients, no significant differences were found in the pooled analysis for overall, disease-free and disease-specific survivals between SLNB and END arms. This is the only available meta-analysis with a subgroup analysis based on three follow-up periods (3, 5 and 10 years) [48]. Only 2 out of 12 studies were randomized controlled trials [42, 43], regarded as high quality studies with a low risk of bias based on 5 domains assessed by the Cochrane risk of bias tool (RoB2) [49].

### *Functional Outcomes of SLNB Compared to END*

Besides the oncological outcomes, treatment-related morbidities are also particularly important for those patients with early oral cancers as their survivorship is expected to be long.

Schiefke et al. [50] retrospectively analyzed post-operative quality of life and functional status in 49 clinically N0 oral & oropharyngeal cancer patients 1–5 years (mean: 26.8 months) after SLNB or selective neck dissection. In this non-randomized study, the SLNB group ($n = 24$) showed superior outcomes in terms of swallowing ($p = 0.043$), neck scar impairment ($p < 0.001$), shoulder pain ($p < 0.012$) and shoulder function on daily activity ($p < 0.001$).

A similar non-randomized retrospective study conducted by Karin et al. [51] reviewed 62 patients for shoulder function status and subjective impairment after SLNB ($n = 33$) or END($n = 29$) also echoed by previous findings. Improved shoulder functional status in the SLNB cohort were illustrated by both higher mean scores in the Neck Dissection Impairment Index (NDII) (99.7 versuss 94.3, $p = 0.0044$) and modified individual relative Constant Score (99.87 versus 96.13, $p = 0.0018$). However, many assessed parameters in the quality of life and clinical assessments after the SLNB and elective neck dissection did not reach statistical significance in these studies.

The retrospective design and small sample size of these studies necessarily reduces the confidence with which we can interpret these results.

The Senti-MERORL trial [45] included prospective data collection of neck-shoulder impairment assessed by the arm abduction test (ATT) and a self-reported questionnaire on quality of life (QoL) [52] after randomization to treatment by SNLB or END. A significantly better shoulder function was reported in the QoL questionnaire among patients with SLNB at 6 and 12 months post-operatively, including less constriction of the neck ($p < 0.01$), less shoulder stiffness ($p = 0.01$), less limitation in the ability to reach above the head for objects ($p = 0.03$), improved ability to undertake leisure activities ($p = 0.03$) and less concern regarding the cosmetic appearance of the neck after the procedure at 12 month post-operatively ($p = 0.04$) [45] .In addition, until 6 months post-operatively, the percentage of patient achieving 180° arm abduction without pain or effort is still significantly higher in the SLNB group (76.29% versus 60.23%, $p = 0.02$) resulting in less physiotherapy prescription compared with END up to 6 months post-operatively (17.86% versus 31.96%, $p = 0.02$) [45].

Hasegawa et al. [44] also concluded that among the 275 randomized patients, SLNB demonstrated similar superior functional performance compared to END. Up to the first year after treatment, the scores of neck functionality in the SLNB group, in term of neck stiffness ($p = 0.001$), constriction ($p = 0.001$), numbness ($p < 0.001$) and shoulder drop ($p = 0.049$), were significantly better than those with END.

## Disadvantages of SLNB

### *Impact on Survival for Patient with Occult Positive Nodal Disease*

Patients with a positive SLNB are required to undergo a second staged operation (completion of neck dissection) followed by adjuvant therapy if indicated. As a result, the additional step of SLNB may introduce a delay in treatment completion compared to upfront elective neck dissection strategy.

McMahon et al. [53] have particularly addressed this issue in a matched pair analysis of node positive early oral cancer patients treated both by SLNB and END. In this study, cases were matched using a specifically designed algorithm based on a number of characteristics, including absence of associated dysplasia, depth of invasion, extranodal extension and perineural invasion, achieving a perfect match in all 38 SLNB patients with the END counterpart. There was no difference in the 5-year overall ($p = 0.958$) and disease specific survival ($p = 0.628$) in the $SLNB^+$ and $END^+$ cohorts. The study concluded there was no survival disadvantage for the $SLNB^+$ group compared with $END^+$ group, however the authors acknowledge further data should be analyzed to confirm these findings.

### *Problems in Identifying a Sentinel Lymph Node Intra-Operatively*

Sentinel lymph node biopsy is technically demanding and requires expertise. It is not readily available universally and requires a well-trained multidisciplinary team to achieve the best results.

The procedure requires pre-operative injection of radiotracer submucosally at the periphery of the primary tumor followed by nuclear medicine imaging. The success rate of correctly identifying sentinel lymph nodes intra-operatively has been widely reported to be more than 95% [53, 54]. In early studies, the primary floor of mouth tumours were shown to have a higher failure for the identification of sentinel lymph node due to the "Shine-through artefact" [55, 56], with around 75% of the sentinel lymph nodes being found in levels I and IIa [18].

It is more challenging to identify sentinel lymph nodes in close proximity to the injection site of the radiotracer although the shine through effect can be minimized by new technology, including pre-operative single-photon emission computed tomography with computed tomography (SPECT/CT), near-infrared fluorescence imaging with indocyanine green (ICG), hybrid optical/radiotracer, and other novel radiotracers such as Lymphoseek.

## *SLNB Technique: State of the Art*

SPECT/CT has an additional advantage over planar images to illustrate the complex lymphatic drainage because of the better delineation of SLB from surrounding structures, especially for the SLN close to the primary tumour [57]. In a retrospective cohort encompassing 41 patients with clinically N0 T1/T2 floor of mouth tumour, SPECT/CT detected an additional 15% of sentinel lymph nodes compared with conventional planar lymphoscintigraphy [58, 59]. In a further study, 3% of clinical node-negative T1/T2 oral cancer patients were upstaged when using SPECT/CT compared to planar lymphoscintigraphy alone [60]. Figure 8.1 showed a SPECT/CT illustrating a SLN in the previously radiated neck.

Optical fluorescent tracers such as (ICG have also shown good reliability in identifying sentinel lymph nodes in the neck. Near-infrared (NIR) fluorescence imaging can be combined with the hand-held gamma probe intra-operatively to identify the SLN after "tracer cocktail injection" of ICG-$^{99m}$Tc-Nanocoll [60]. Christensen et al. [61] detected an additional 12% SLNs (11 out of 94) in 30 T1/T2 oral cancer patients by using this combination of optical- and radiotracer. Figure 8.2 showed a SLN only illustrated by near-infrared image intra-operatively.

A new radioactive agent, $^{99m}$Tc-tilmanocept (Lymphoseek ®, Navidea Biopharmaceuticals, Inc.) [63], is targeted to a small sized receptor (CD206) on SLN. It has a property of rapid clearance from the injection site, rapid uptake and high retention rate within the SLN and a low uptake rate by other lymph nodes. A

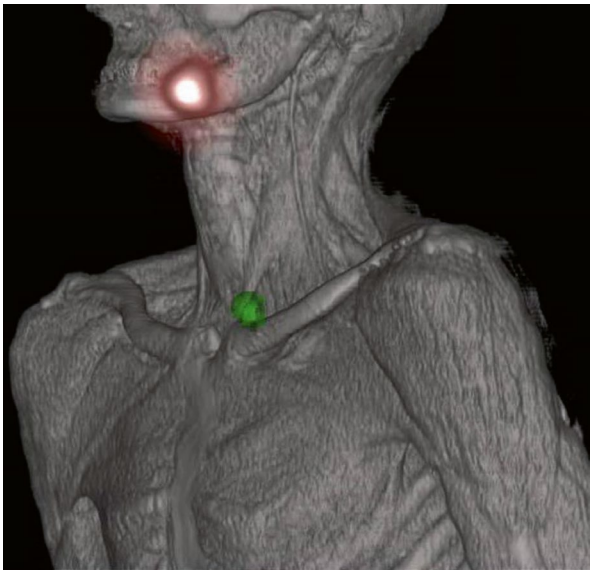

**Fig. 8.1** A 3-D rendered SPECT/CT showing a patient with a second primary tumor arising from the left floor of mouth (*red spot*). SPECT/CT shows SLN at the left level IV/V (*green spot*) in the previously operated neck

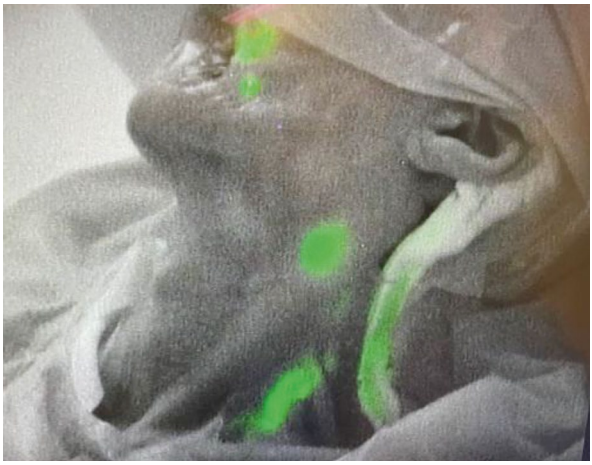

**Fig. 8.2** Screen grab intraoperative near-infrared (NIR, PDE—Hamamatsu ®) image by ICG-Nanocoll and 99mTc-Nanocoll and injected at two time points [62]. SLN identified at left level IIa (confirmed with hand-held gamma probe). The second echelon nodes at the left level V were only illustrated by NIR image (No gamma probe signal detected)

phase III multi-institutional prospective trials recruiting T1-T4 head and neck SCC patients of which 92.9% were oral tumours reported a low false negative rate (FNR) in using Lymphoseek ® in the SLNB (2.56%, 95% CI: 0.06–13.49%) [64].

It should be noted that additional equipment to improve the accuracy of SLNB has the potential to reduce the cost effectiveness of SLNB.

## Special Consideration for the Primary Floor of Mouth Tumor

Particular concern regarding cervical metastasis has been raised for the primary tumours of the floor of mouth and this group was specifically highlighted by the European Association for Cranio-Maxillo-Facial Surgery (EACMFS) position paper in 2020 [65]. According to these guideline recommendations, FOM tumours should be approached differently from other oral cavity cancers in case of DOI > 1.5 mm because of the higher risk of regional propensity of early metastasis.

However, evidence is conflicting in the literature. An early retrospective cohort conducted by Kunzel et al. [66] only showed 4.8% pathological T1 FOM tumor harbouring occult cervical metastasis. It is much lower than the generally accepted threshold of 15%–20% risk of occult metastasis as an indication for elective neck dissection [67]. To understand this controversy further, we undertook a retrospective cohort study comprising of 847 early tongue and floor of mouth cancer patients (unpublished data) staged by SLNB from 2005 to 2021. The occult metastatic rate of FOM was not higher than those of the tongue (T1: 15.5% versus 29.0%, $p = 0.45$; T2: 29% versus 48%, $p = 0.03$). At a minimum follow-up period of 2 years, there was no significant difference in disease free survival ($p = 0.31$). Further prospective high-quality studies are still warranted to solve this debated issue.

## Current National Guidelines on the Management of Clinical N0 Neck

Although around 40% to 50% of oral cavity cancers present as an early disease, there is still no global consensus on the management of clinical N0 neck. The aforementioned 2020 EACMFS position paper undertook a comprehensive review on this issue [65] and commented that the most explicit recommendation on the current management of clinical N0 early oral cavity cancer are the National Comprehensive Cancer Network® (NCCN®) [68, 69] and the American Society of Clinical Oncology (ASCO) [30] guidelines.

In oral cavity cancer, the significance of oral cavity tumor's DOI in the prediction of nodal metastasis and survival outcome has been fully reflected by the incorporation of DOI into the American Joint Committee on Cancer (AJCC) eighth edition cancer staging system [70]. NCCN guideline in 2019 first introduced SLNB as an option of managing the neck in a clinical N0 T1-T2 oral cavity cancer based on multiple single and multi-institutional trials providing adequate evidence to show comparable outcomes between SLNB and END. However, noting at the same time, a lack of direct comparison between SLNB and END, which has since been addressed [68].

In the latest version of the NCCN guidelines [69], both the SLNB and tumour DOI are regarded as adequate predictors for occult cervical metastasis. In the guidelines, END is strongly recommended by the panel for tumour with DOI more than 3 mm if radiotherapy is not planned supported by D'Cruz et al.' randomized trial [14]. For a DOI between 2 and 4 mm, the recommendation is individualized based on clinical and tumour characteristics [69].

The most recently published guideline (2024) by the American Head and Neck Society (AHNS) [71] offers a more comprehensive recommendation on the surgical management of the neck. In T1 primary tumours of the tongue and floor of the mouth with DOI more than 2 mm, it is recommended to proceed to END as at least 20% of them have pathologically node-positive disease. For tumours staged as T2 or above with clinically N0 disease, END is also recommended. However, the guidelines state that SLNB can also be considered in the scenario of clinically node-negative T1/T2 oral cavity cancer, essentially leaving the choice on the treating clinician. The guideline also emphasized the need of a well-trained multi-disciplinary team and the use of SPECT/CT and gamma probe detection in order to provide the most reliable SLNB procedure.

Although these guidelines recommend using tumour DOI to guide the decision on neck management, it should be noted that in practical terms, the accurate measurement of the DOI is challenging and only definitively confirmed by histopathological assessment of the primary tumour excision. This challenge has been retrospectively reviewed by Cocker et al. [72] in 185 clinical N0 oral cancer patients who underwent different pre-operative imaging modalities. Only 56.7% of MRI and 57.1% of CT could achieve the accuracy to within 3 mm of the histopathological measurement of tumor depth. Intra-operative ultrasound (IOUS) appeared to be the

best imaging modality, but only 78.2% achieved that accuracy. That finding particularly highlighted the problem of using pre-operative assessment of tumour thickness by imaging to stratify treatment decision according to different guidelines as only 82% and 50% of oral cancer cases were correctly staged pre-operatively as T1 and T2 tumors, respectively.

## Ongoing Trials

A multi-center prospective phase II/III randomized controlled trial led by Lai et al. is currently ongoing in North America (NCT04333537) [73]. It aims to recruit 618 cT1-2N0M0 oral SCC patients randomized to SLNB or END arms. Parameters including neck and shoulder function, overall and disease-free survivals, pattern of treatment failure, and quality of life will be evaluated. It is hoped these results will provide the definitive evidence-based treatment strategy for early oral cancer without clinical lymph node involvement in the neck.

## Is Removal of Non-cancer Affected Lymph Nodes Harmful?

In case of nodal positive disease, active treatment is generally believed to be beneficial. However, with the emergence of immunotherapy, elective nodal treatment strategy might not be the optimal one. Recent murine studies [74] have shown surprising findings on the impact of regional lymphoablative procedures on the host immunotherapy response. In these animal models, the initial lymphatic preserving treatment can potentiate the immunotherapeutic response by mobilizing the systemic anti-tumour immunity and eventually controlling the regional and distant metastatic disease. Conversely, mice that underwent an early elective lymph node removal fared worse than the control group [74]. If these results are confirmed in human studies, there may be a treatment paradigm shift to preserve unaffected lymph nodes.

## Conclusion

Sentinel lymph node biopsy is an accurate technique in assessing the regional lymph node status in centers with expertise and is of particular value for head and neck cancer due to the significant impact of regional metastasis on survival outcomes.

The current evidence mentioned in this chapter has illustrated comparable oncological outcomes with reduced morbidity profile in SLNB compared with the traditional END.

SLNB is a reasonable individualized approach in stratifying treatment for patients with clinical node-negative early oral cavity. With technological advancements, the results of ongoing prospective randomized trials as well as the new insight of interaction of cancer and immune cells, the role of SLNB in the head and neck cancer treatment will be further refined.

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# Chapter 9
# Partial Laryngeal Surgery in 2023

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## Introduction

Since the pioneering of total laryngectomy (TL) in 1873, the surgical approach to treating advanced laryngeal cancer (LC) has evolved significantly, emphasizing laryngeal preservation and functionality over the past 150 years [1, 2]. Originally, treatments prioritized loco-regional tumor control, with functional conservation being less important. However, a deeper understanding of LC's natural progression and epidemiology led to a shift in focus. The late twentieth century saw peak interest in partial laryngeal surgeries, particularly for early- and select intermediate-stage cancers, which helped preserve essential functions like speech, breathing and swallowing [3–6].

As the twentieth century ended, this optimism waned due to new chemoradiation protocols aimed at organ preservation, which promised better disease control and improved patient quality of life, even for more advanced stages [7, 8]. Despite initial enthusiasm, subsequent studies revealed limitations in survival rates, prompting a reassessment of the non-surgical strategies [9]. Recently, there has been a renewed interest in partial laryngeal surgeries, spurred by advances in understanding tumor biology, patient selection, surgical techniques and tailored surgical responses to tumor size [10–12].

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In this landscape, open partial horizontal laryngectomies (OPHLs) have become key in modern efforts to maintain laryngeal function. These surgeries have been validated over recent decades for effectively managing intermediate to locally advanced LC without extensive neck metastases [13].

## Key Clinical and Pathological Issues for Preserving Surgical Function of the Larynx

Modern partial laryngeal surgery has advanced by leveraging the unique clinico-pathological features of tumors, broadening the scope of surgical intervention to encompass intermediate and advanced T stage tumors. This progression enables a greater number of patients with advanced disease to undergo surgeries that prioritize the preservation of laryngeal functions. Several critical factors underpin this approach:

1. **Comprehensive patient selection**. Selecting patients for partial laryngeal surgery goes beyond tumor characteristics to include a detailed assessment of clinical and demographic factors. This holistic approach helps pinpoint candidates who can handle the rigors of surgery and are likely to recover effectively, all while maintaining vital laryngeal functions. Factors to consider include the patient's overall health, age and potential for recovering from surgery-related complications [14].
2. **Patterns of local tumor spread in advanced LC**. The progression patterns of T3 and T4a LC are distinctive—T3 tumors penetrate deeper into the laryngeal tissues, while T4a tumors extend beyond the larynx into surrounding structures. Recognizing these patterns pre-operatively is vital for planning of precise surgeries that selectively target affected laryngeal areas while conserving crucial anatomical structures [15, 16].
3. **Reduced metastatic risk in advanced glottic cancer**. Advanced glottic laryngeal cancers typically have a lower risk to metastasize to the cervical lymph nodes than supraglottic ones. This feature is crucial for opting for surgical methods that focus on conserving laryngeal functionality, allowing for surgical treatment alone even at advanced stages. Such strategies significantly enhance quality of life by maintaining voice and swallowing functions [17].
4. **Differentiation of arytenoid fixation patterns**. Although arytenoid cartilage fixation was traditionally seen as a negative indicator for partial surgery, recent analyses suggest some patterns might still be conducive to successful laryngeal preservation. Identifying these patterns enables more accurate and safer patient selection for surgery [18].
5. **Pre-operative stratification based on topography**. The technique of classifying advanced tumors pre-surgery based on their topographical spread, especially using the "magic plane" concept, marks a substantial advancement. This strategy, focusing on tumor invasion into the posterior paraglottic space and considering arytenoid mobility, enhances surgical precision and expands the possible surgical directions, thus improving laryngeal preservation outcomes [13, 19, 20].

## Recent Developments in Clinical and Radiological Assessments

The progress in treating locally advanced LC highlights the crucial importance of comprehensive endoscopic and radiological evaluations. Recent enhancements in endoscopic techniques include the adoption of high-definition videolaryngoscopy, narrow-band imaging (NBI), and the STORZ professional image enhancement system (SPIES) endoscopy. These tools provide detailed images of the mucosal surface and vascular structure, crucial for an early detection and precise delineation of neoplastic lesions, especially around their margins [21, 22].

In the management pathway for an OPHL, in-office endoscopy is pivotal. It assesses the superficial spread of the tumor, its potential pathways of extension, and the mobility of the vocal cords and arytenoids [18, 23]. Imaging techniques like computer tomography (CT-scan) and magnetic resonance imaging (MRI) are essential for visualizing the submucosal progression of the disease [24]. CT-scans are favored for their quick imaging, high resolution, and ability to assess cartilage involvement and extralaryngeal tumor spread, all key for surgical planning. MRI, with its superior contrast for soft tissues, excels in evaluating submucosal spread and involvement of critical areas like the paraglottic and pre-epiglottic spaces [25, 26]. Adding diffusion-weighted imaging (DWI) to MRI further enhances its utility by distinguishing tumor from peritumoral edema, aiding in the precise planning of conservative surgeries like OPHL, especially in salvage scenarios for radio-recurrent tumors [27, 28].

A thorough endoscopic assessment under general anesthesia finalizes the preoperative work-up, offering a comprehensive analysis of less visible growth patterns of the tumor, enabling lesion palpation and targeted biopsies. This step is integral for grasping the three-dimensional aspects of the tumor and planning the type of surgery required within a modular approach.

Effective collaboration between clinicians, radiologists and pathologists is indispensable, especially in complex cases. This teamwork should be a dynamic, cyclic process of precise inquiries and targeted responses to refine diagnostic and therapeutic strategies [29]. This collaborative approach ensures that each surgical plan is tailored to the patient's specific condition, enhancing both the precision and efficacy of the treatment.

## Innovations in Surgical Techniques for LC

Some studies emphasize the necessity for advancing the conventional methodologies of partial laryngeal surgery to treat progressively complex, locally advanced tumors. This development underlines the importance of adopting flexible, modular surgical methods that ensure complete tumor excision, even in intricate scenarios [30]. The surgical arsenal for advanced laryngeal cancer has been substantially enhanced by integrating supratracheal partial laryngectomy (STPL), a notable

progress in addressing glottic tumors that extend to the anterior, inferior, and/or posterior subglottic areas, including cases prone to extralaryngeal dissemination. This addition expands the range of surgical interventions available, providing a holistic solution to particularly challenging conditions [31, 32].

Historically, the primary surgical options to select for locally advanced supraglottic and glottic tumors, especially those with transglottic spread, have been supraglottic and supracricoid laryngectomies. The introduction of STPL aims to increase the thoroughness of surgical procedures in difficult tumor sites, improving the chances of complete tumor removal while attempting to maintain maximum laryngeal functionality [33].

The establishment of STPL and its proven success in both oncological and functional outcomes have led to the adoption of a new classification by the European Laryngological Society (ELS) [34]. This classification system divides OPHLs into three types, depending on the lower boundary of the surgical resection, each designed to conserve laryngeal function: type I (supraglottic laryngectomies), type II (supracricoid laryngectomies), and type III (supratracheal laryngectomies). Each type can extend to adjacent laryngeal and pharyngeal structures, involving one arytenoid (+ARY), the base of the tongue (+BOT), the piriform sinus (+PIR) and the cricoarytenoid unit (+CAU). OPHLs type II and type III may or may not preserve the epiglottis (indicated by suffix A or B). This system was devised to support a conservative, adaptable surgical approach to treating laryngeal cancer, offering surgeons a choice among twelve distinct procedures that balance oncological success with quality of life preservation [30].

In specific cases, the narrow margins of resection obtained from these surgeries, if confirmed disease-free through a detailed and standardized evaluation of the surgical sample, are adequate to provide positive oncological results, even for locally advanced tumors [35].

Furthermore, in the management of these tumors, it's vital to consider the heightened risk of lymphatic metastasis to level VI, notably in tumors with subglottic extension and anterior extralaryngeal spread, which necessitates critical VI level dissection [36]. Moreover, due to the potential for unrecognized extralaryngeal extension in advanced cases, the removal of strap muscles is essential to achieve a degree of radicality comparable to TL, a technique proven by Schindler and coll. to preserve swallowing function [37].

## Oncologic Outcomes Following OPHLs

OPHLs have significantly improved the conservative management of advanced LC, achieving stable and impressive results. These treatments are particularly effective when the tumors are confined within certain anatomical boundaries (the "magic plane") without causing arytenoid immobilization. This underscores the importance of understanding anatomical and functional divisions for predicting better outcomes in patients, a method that proves more insightful than the traditional TNM staging

system, emphasizing the importance of tumor positioning in treatment strategies. Initial studies indicated that posterior T3 tumors that invade the posterior paraglottic space and cause arytenoid immobilization are less responsive to OPHLs, leading to poorer oncologic results compared to anterior tumors [19]. Further research confirmed these observations, showing that survival rates for T3–T4a tumors treated with OPHLs are significantly better for anterior tumors compared to posterior ones, with notable differences in survival statistics. These insights, combined with a variety of surgical options, reveal a distinct prognostic advantage [20].

Another recent study showed that OPHLs can still be effective for tumors extending posteriorly and causing arytenoid immobilization, especially if the subglottic extension at the vocal cord's midline is under 10 mm [18]. Analyzing data from reviews on treatment-naïve patients with T3N0 LC, who demonstrate high local and loco-regional control and impressive 5-year overall survival rates, has become more understandable (Tables 9.1 and 9.2). Campo and coll. highlighted that OPHLs serve as an effective treatment for naïve pT3N0 LC patients. Additionally, the ability of OPHLs to maintain high rates of survival free from laryngectomy and laryngo-esophageal dysfunction in T3 patients highlights its capacity to balance oncological effectiveness with quality of life considerations [38].

The conservative approach to managing T4a tumors, often seen as limited to cases with minimal extralaryngeal tumor extension initially staged as cT3 and later identified as pT4a in pathological findings, has gained support from recent multicenter studies [39]. This strategy, particularly using type II and III OPHLs for T4a tumors with minimal extralaryngeal volume, aligns oncological outcomes with those of less advanced T–categories. The frequent understaging during clinical assessments highlights the effectiveness of a systematic and structured approach in managing these more complex and inherently riskier cases.

**Table 9.1** Studies analyzing 5-year oncologic outcomes following OPHL for T3 LC

| Authors and year | No. of patients | OS (%) | DSS (%) | DFS (%) | LRC (%) | LFS (%) |
|---|---|---|---|---|---|---|
| Laudadio et al. (2006) [40] | 58 | 88.7 | NA | 77.6 | NA | NA |
| Sánchez-Cuadrado et al. (2011) [41] | 17 | 52 | 64 | 67 | NA | NA |
| Mercante et al. (2013) [42] | 32 | 87.3 | NA | 78.2 | 96.2 | NA |
| Sperry et al. (2013) [43] | 34 | 83.8 | 84.4 | NA | 85.2 | 92.6 |
| Rizzotto et al. (2015) [32] | 50 | 86.0 | NA | 86.0 | 86.0 | NA |
| Succo et al. (2016) [12] | 442 | 87.8 | NA | 87.9 | 89.7 | 93.3 |
| Succo et al. (2018) [19] | 390 | 90.1 | 94.5 | 87.4 | 88.8 | 86.8 |
| Xia et al. (2018) [44] | 106 | 65.8 | 73.6 | 72.1 | NA | NA |
| Del Bon et al. (2019) [20] | 67 | 74.1 | 80.5 | 63.4 | NA | 63.8 |
| Gong et al. (2019) [45] | 42 | 77.8 | 77.8 | 63.3 | NA | NA |
| Mattioli et al. (2021) [46] | 28 | 92.9 | 100 | 89.3 | NA | 89.3 |
| De Vincentiis et al. (2022) [13] | 116 | 79.3 | 85.3 | 81 | NA | 82.76 |

*DFS* disease-free survival, *DSS* disease-specific survival, *LFS* laryngectomy-free survival, *LRC* locoregional control, *NA* not available, *OS* overall survival

**Table 9.2** Studies analyzing 5-year oncologic outcomes following OPHL for T4 LC

| Authors and year | No. of patients | OS (%) | DSS (%) | DFS (%) | LRC (%) | LFS (%) |
|---|---|---|---|---|---|---|
| Laudadio et al. (2006) [40] | 13 | 61.5 | NA | 53.8 | NA | NA |
| Rizzotto et al. (2015) [32] | 51 | 80.4 | NA | 60.8 | 62.7 | NA |
| Succo et al. (2016) [12] | 113 | 71.2 | NA | 68.1 | 71.7 | 93.3 |
| Succo et al. (2018) [19] | 89 | 81.9 | 91.3 | 71.2 | 75.5 | 72.9 |
| Del Bon et al. (2019) [20] | 18 | 71.8 | 71.8 | 43 | NA | 43.1 |
| De Vincentiis et al. (2022) [13] | 33 | 70.9 | 77.4 | 77 | NA | 66.67 |
| Succo et al. (2023) [39] | 134 | 82.1 | 89.8 | 75.7 | NA | 93.3 |

*DFS* disease-free survival, *DSS* disease-specific survival, *LFS* laryngectomy-free survival, *LRC* locoregional control, *NA* not available, *OS* overall survival

## Assessment of Functional Outcomes After Partial Laryngeal Surgery

OPHL is recognized as a critical conservative surgical method for treating LC, spanning from the early and intermediate stages to certain advanced T4a LC [34]. These surgeries are noted for their effectiveness in cancer control, evidenced by high rates of overall survival (OS), disease-free survival (DFS), and laryngectomy-free survival (LFS) rates [12, 47]. Furthermore, OPHL aims to preserve the larynx's essential functions, such as swallowing, breathing, and speaking and reduce the likelihood of needing a permanent tracheostomy. Five-year rates of maintaining laryngeal function after OPHL are reported to be between 91.2% and 98.5%, depending on the initial severity of the disease, with types II and III OPHL showing similar functional outcomes [33, 37, 48].

Assessing functional results after OPHL is crucial for surgical planning and involves detailed preoperative analysis of patient-specific and disease-specific factors to develop personalized treatment plans. Recovery of voice, breathing, and swallowing functions is generally good, though there is notable variation. Type I OPHL typically results in better voice quality due to less impact on the vocal folds, while types II and III often experience a considerable reduction in voice quality, although rehabilitation techniques can help achieve effective verbal communication [49].

Post–OPHL, regaining swallowing ability poses a significant challenge, with initial post-surgery issues improving over 6 months, enabling most individuals to resume normal eating. Still, ongoing swallowing difficulties may increase the risk of complications like aspiration pneumonia. Differences in the duration of tracheal cannula usage, nasogastric tube (NGT) feeding, and hospital stays illustrate the variability in recovery as supported by existing studies [37, 50].

Recent research has highlighted favorable laryngeal function preservation rates, with a focus on identifying predictors of challenging recoveries to enhance pre-surgery patient selection [12, 47]. Factors such as older age, lower body mass index (BMI), smoking, existing health issues, osteophytosis, and advanced cancer stages have been linked to poorer health and functional outcomes post-surgery [14].

The focus has increasingly shifted towards not only the oncological results of partial laryngectomies but also their functional impacts, emphasizing the importance of quality of life post–OPHL. Recent literature underscores this interest, aiming to improve both survival and post-operative quality of life. Despite some initial disappointments in functional recovery, there are many therapeutic and surgical options available that can significantly enhance essential laryngeal functions [51, 52].

Recent advancements have shown significant potential for functional improvement through specific interventions, such as phonosurgical injection techniques to aid voice and swallowing, and transoral laser microsurgery for managing laryngeal stenosis to improve breathing. The PROEL (proprioceptive elastic) method is also being utilized for voice rehabilitation, showing promising early results in phonatory recovery [53]. These varied approaches highlight the comprehensive efforts to improve and manage post–OPHL functional recovery, focusing on both addressing physical deficits and enhancing remaining capabilities, customized to each patient's unique situation.

## Management of LC Recurrence Post Radiotherapy or Laser Treatment

Research into the various surgical methods available after initial treatments like radiotherapy (RT), chemoradiation (CRT), or transoral laser microsurgery (TOLM) has become increasingly crucial. Historically, TL was the primary technique for managing treatment failures. Currently, however, the success of less invasive surgical methods in specific situations is acknowledged. These less invasive methods are proving to be effective alternatives to TL, providing both cancer control and maintaining laryngeal functions.

An extensive review of multiple studies shows that despite early detection of recurrences, approximately 30% of patients exhibit advanced disease in the tissue samples examined [12]. This highlights a common problem of mis-staging in these recurrent scenarios. Factors contributing to this include the biological nature of tumors that recur after radiation—hidden disease extent due to chronic swelling and scarring, more aggressive tumor growth, increased chances of spread beyond the larynx, and more frequent occurrence of undifferentiated tumors with invasion into blood vessels and nerves—and changes in anatomy and structure post–laser surgery [54–56].

OPHLs are now recognized as a viable surgical choice for recurring LC, suitable for a wide array of clinical situations. These include rT1 and rT2 tumors that are difficult to assess endoscopically or that extend across the commissure, rT2 tumors with reduced movement of the vocal cords, and rT3 tumors with limited impact on the spaces next to or in front of the glottis, including those affecting the thyroid cartilage without spreading outside the larynx [57].

Studies assessing the effectiveness of OPHL after RT and TOLM show local control (LC) rates of 70–95% after 2 years, DFS rates of 70–90% over 3 years, and OS rates of 70–90% over 5 years [58]. Even with these promising figures, some patients may still need a salvage TL. The rate of laryngeal preservation is reported at 85.2%, with a high success rate in removing breathing tubes, signifying effective airway management. Yet, laryngeal narrowing remains a significant complication. Most patients report good swallowing ability, although some require a feeding tube. Vocal results vary, with some experiencing major changes in voice quality [58].

In conclusion, for certain recurrent LC cases, OPHLs provide a treatment option that effectively balances cancer treatment with functional preservation, advocating for its wider use in clinical settings.

## Discussion

The resurgence in partial laryngeal surgery, particularly directed at treating intermediate to advanced stages, is due to a better grasp of the disease's progression, the minimal occurrence of cervical metastases in glottic cancers, and the established spread patterns of LC. This knowledge has enhanced the application of OPHLs, which can provide outstanding oncological results with a one-time intervention if surgical completeness is verified through pathological examination. Recent developments in the selection of patients and tumors go beyond TNM classifications by integrating endoscopic, functional and imaging assessments, thereby improving the safety and effectiveness of conservative treatments at advanced stages. Innovations in surgery, such as the adoption of extended partial procedures like STPLs for tumors that approach the subglottic area and pose a high risk of extending beyond the larynx, have facilitated a modular surgical approach, increasing the variety of cases that are amenable to such surgeries. An improved understanding of areas prone to recurrence, particularly regional recurrence at the VI level, has improved loco-regional management with more precise and targeted surgical methods and planned dissections. There is a notable variation in functional outcomes after partial laryngeal surgeries, especially concerning voice quality and respiratory functions, which highlights the complexities of recovery after surgery. This variation emphasizes the urgent need for ongoing research into rehabilitation methods to improve these outcomes. While advanced surgical methods and post-operative treatments are designed to conserve as much functionality as possible, patient outcomes can still differ widely. Corrective surgeries, like injection laryngoplasties, are increasingly essential for enhancing functional results, particularly in terms of voice and breathing efficiency. There are ongoing debates regarding adjuvant treatments for advanced pT stage tumors, particularly about how post-operative radiotherapy might adversely affect functional outcomes without clearly benefiting oncological results in cases with positive margins [59].

## Conclusions

Based on existing research and a detailed analytical review, it can be concluded that today OPHLs are pivotal in the conservative treatment of intermediate to advanced stages of LC. These procedures are competitive with non-surgical organ preservation methods in terms of oncological results and maintaining laryngeal functionality. Choosing suitable candidates for OPHLs is of utmost importance. Currently, surgeons have access to more precise parameters that aid in understanding and improving this intricate decision-making process, which largely depends on the physician's expertise. The main goal is to completely remove the cancer with one therapeutic approach, positioning OPHLs as a key strategy within targeted treatments. The necessity of turning to multimodal treatments due to unanticipated progression of the disease is seen as a limitation of single-mode surgical approaches that aim to preserve the function of the larynx. This emphasizes the need for thorough pre-operative evaluations and highlights the challenges of treating advanced cases, with the objective of retaining organ function while ensuring oncological safety.

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# Chapter 10
# Managing the Internal Carotid Artery in Head and Neck Cancer: Where Are We?

**Alessandra Ruaro, Stefano Taboni, Marco Ferrari, and Piero Nicolai**

## Introduction

Management of carotid artery (CA) involvement, with special reference to the common (CCA) and internal carotid artery (ICA), in head and neck cancer (HNC) has been historically debated. Controversies were basically related to the high risk of complications of treatments, which were not counterbalanced by a reasonable control of the disease. On the other hand, it is well known that infiltration of the CA wall by cancer, if untreated, invariably leads to fatal hemorrhage or thrombosis of the vessel with possible brain ischemia.

The surgical approach to CCA/ICA involvement has profoundly evolved over the years. Historically, resection of cancers invading the carotid axis was not considered feasible. In the 1990s, unilateral resection of the CCA and/or the ICA was proposed for selected patients with HNC [1]. However, in 2002 the American Joint Commission on Cancer defined CCA/ICA encasement or invasion in the T4b category as "unresectable" at that time [2]. Subsequently, several authors advocated that CCA/ICA resection was associated with potentially better local control with an acceptable risk of neuromorbidity and mortality [3–15]. Starting from 2021, the National Comprehensive Cancer Network guidelines (version 2.2021) stated that the T4b category (which includes CCA/ICA encasement) was "not an absolute

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contraindication to resection in selected patients in whom total cancer removal is possible".

In addition to surgical management, non-surgical methods have been explored, and data on survival outcomes with surgical and conservative approaches have been reviewed [16, 17]. Nevertheless, these data should be cautiously interpreted in view of the small number of patients in most series, limited follow-up, and differences in patient selection criteria in relation to the approach. In this review, attention will be paid to analyze the evolution over the years of the definition of CCA/ICA involvement, the different impact of surgical and nonsurgical approaches, and prognostic outcomes.

## Carotid Artery Encasement: Definition of the Clinical Scenario

CA involvement has a low incidence, presenting in 2–7% cases of advanced HNCs [18]. CCA/ICA walls can be encircled or further invaded by HNC, either by a primary tumor or nodal metastases with extra-nodal extension.

Clinical presentation can vary, with the CA being displaced, surrounded by the mass, or with its wall invaded in different degrees of depth (Fig. 10.1). Preoperative imaging is critical in the decision-making process, even though standardization in terms of radiological criteria that define CA involvement are lacking. Radiologic assessment through magnetic resonance (MR) has been demonstrated to be reliable in predicting CA involvement [18], whereas the role of computer tomography (CT) has been doubted [19]. During the '90s, the analysis on 49 MR conducted by Yousem et al. revealed that when tumor surrounds the CA for more than 270°,

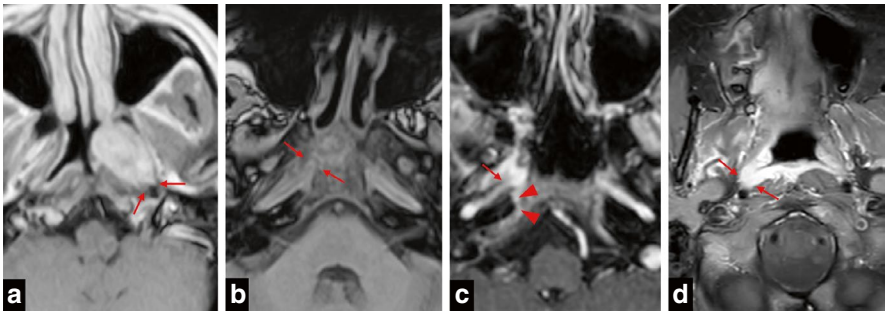


**Fig. 10.1** Examples of malignancies abutting/invading the internal carotid artery; (**a**) nasopharyngeal salivary polymorphous adenocarcinoma encasing the parapharyngeal internal carotid artery for roughly 90° (*arrows*); (**b**) recurrent nasopharyngeal carcinoma encasing the anterior genu of the internal carotid artery for almost 180° (*arrows*); (**c**) nasopharyngeal adenoid cystic carcinoma invading the horizontal petrous internal carotid artery (*arrow*) and petroclival junction (*arrowheads*); (**d**) nasopharyngeal adenoid cystic carcinoma abutting the parapharyngeal internal carotid artery (*arrows*)

invasion of its wall was identified at surgery in 71% of patients [20]. Subsequently, Pons et al., reported that a combination of different elements such as deformation of the CA, encasement for more than 180°, and segmental obliteration of the fat separating the vessel from the tumor was highly predictive of ICA wall invasion [21]. The isolated finding of >180° encasement or fat obliteration was not reliable enough to infer CA invasion. Even if similar findings were concurrently reported by other authors, radiologic criteria to unequivocally define CA involvement remain debated.

## Management and Complications

Consensus on the management of CCA/ICA involvement has not yet been achieved. While some data support the feasibility of CCA/ICA unilateral resection in accurately selected patients with skull base cancer (SBC) [12–15, 22–24] or HNC [3, 4, 6–11, 22, 25, 26], debate arises from the potential severe morbidity (25–60%) [12–14, 24] of resection and reconstruction, neurological complications after vessel removal (12.5–33%) [12–15], and dismal prognosis (2-year OS 11–50%) in advanced-stage malignancies. While CA-sparing alternatives exist and are selectively adopted, these are not devoid of risks and may be associated with unfavorable oncologic results.

When approaching head and neck malignancies with CCA/ICA involvement, accurate evaluation of surgical and non-surgical alternatives is mandatory. In resectable SBC or HNC with CA involvement, complete tumor resection, which may require sub-adventitial dissection or CA resection according to the degree of involvement, is considered to be associated with the highest chance of cure. Of note, the natural history of locoregionally uncontrolled CA-abutting HNC/SBC may lead to the dismal events of vessel rupture and subsequent fatal bleeding. Thus, awareness of the different options is critical, even when a cancer is associated with substantial likelihood of distant recurrence. In this overtly complex setting, clinicians should focus on a few specific questions: is the CCA/ICA resection feasible? Is vessel reconstruction feasible? How accurately can we predict tolerance to CA resection? Is the risk/benefit ratio acceptable? Are there valuable alternatives?

### *Is Vessel Resection Feasible?*

Feasibility of CA resection closely depends on the overall extension of the tumor alongside with involvement of other critical structures. When CCA and parapharyngeal ICA are the only critical structures involved by HNC, resection can be achieved through proximal and distal vessel control and subsequent *en bloc* excision [4, 6, 8–11, 26]. In contrast, distal ligation is hard to be achieved when ICA is invaded within the cranial base. Thus, resection must be performed following preoperative vessel occlusion (Fig. 10.2). Of note, CCA/ICA involvement is rarely the only

critical extension, with CA-invading/encasing HNC/SBC often infiltrating other critical structures such as the prevertebral muscles, spine, mediastinum, dura mater, dural sinuses, and/or brain, thus making complete tumor removal either unfeasible or associated with unacceptable morbidity. Hence, CCA/ICA ablation is justified when there is a reasonable chance of achieving a clear resection margin. Surgeons embarking in such a demanding procedure should be cautious to avoid overfocusing on CA involvement while overlooking other extensions that substantially prevent complete tumor excision. Since the density of critical structures is higher in the skull base, SBCs with involvement of the ICA represent an unparalleled challenge and are frequently unamenable to complete resection. Assuming that ICA resection enables complete tumor removal with adequate margins, different routes (*i.e.* transnasal, transorbital, and transpetrosal approach) provide sufficient exposure to perform an internal carotidectomy [27] (Fig. 10.3).

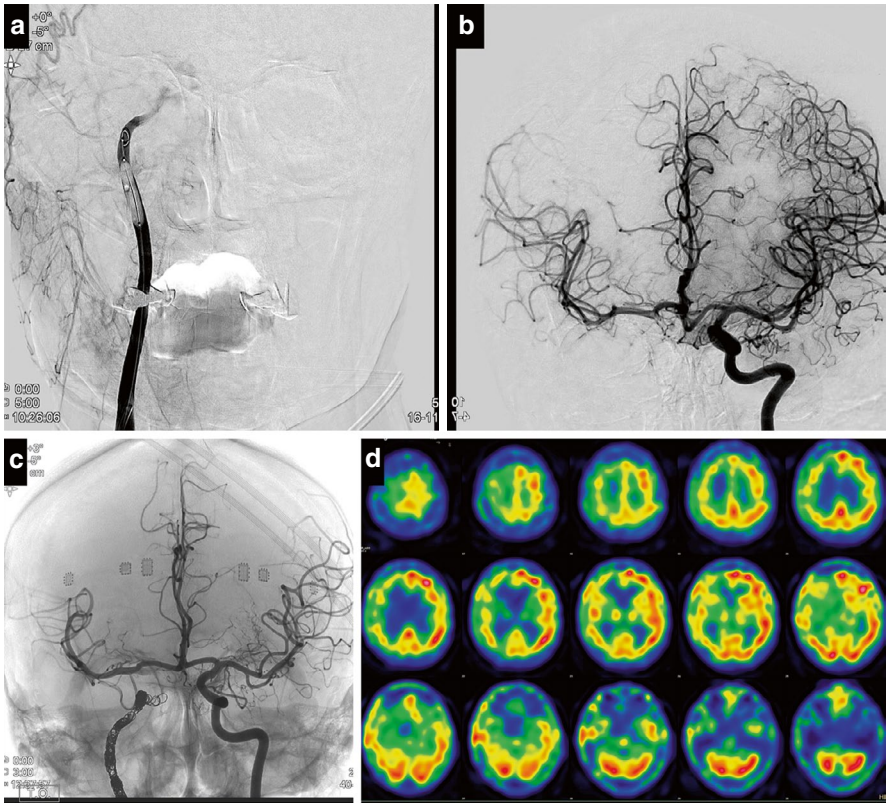


**Fig. 10.2** Internal carotid artery balloon occlusion test; (**a**, **b**) balloon inflated into the right internal carotid artery and catheterization of the left internal carotid artery showing bilateral brain arteriography; (**c**) coiling-based occlusion of the right internal carotid artery with catheterization of the left internal carotid artery showing bilateral brain arteriography; (**d**) single-photon emission computed tomography after right internal carotid artery occlusion, showing mildly reduced perfusion in the right brain lobe

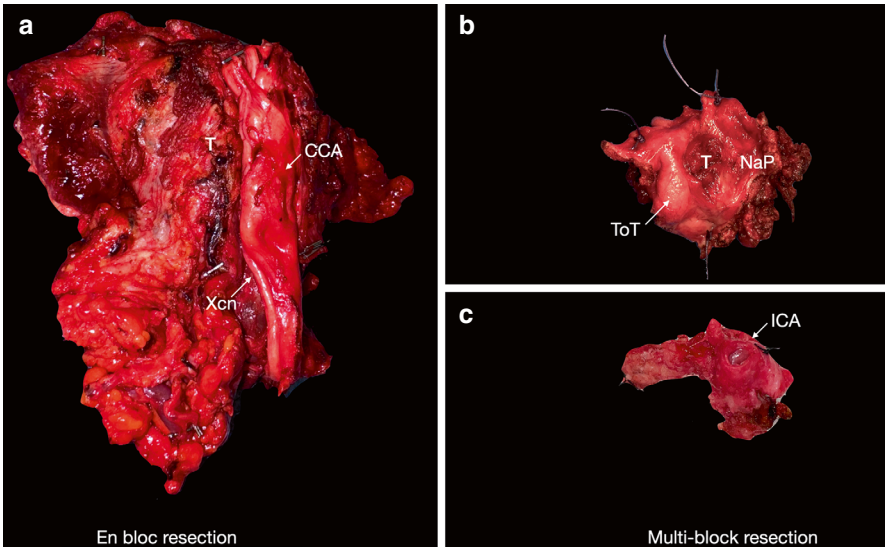


**Fig. 10.3** Examples of surgical specimens of carotid artery-including ablations; (**a**) *en bloc* resection specimen of a nodal metastasis invading the common-internal carotid artery axis; (**b**) specimens of a multi-block, internal carotid artery-including nasopharyngectomy. CCA, common-internal carotid artery axis; ICA, internal carotid artery; NaP, nasopharyngeal posterior wall; T, tumor; ToT, torus tubarius; Xcn, vagus nerve

## *Is Vessel Reconstruction Feasible?*

Starting in the '60s, several techniques have been developed to achieve cerebral revascularization after resection of CCA/ICA. While poor results were seen in the earliest experiences [28, 29], subsequent series reported a substantial improvement in outcomes [3, 30]. Strategies for cerebral revascularization include CA reconstruction, especially in case of lesions involving the CCA or the extracranial segment of the ICA, or bypass surgery, mostly adopted in the revascularization following resection of complex SBC [31].

The available CA reconstruction methods include the use of synthetic prostheses (*e.g.* polytetrafluoroethylene) [26] or autologous graft, such as the greater saphenous vein [3]. In 2008, Miao et al. published a series of 13 reconstructions using expanded polytetrafluoroethylene prostheses and demonstrated its feasibility. In their series, one patient developed postoperative carotid blowout (CB); however, neither persistent neurologic deficit nor graft occlusion were observed [26]. One of the first studies reporting on the use of the greater saphenous vein graft after CCA/ICA removal was conducted by Wright et al. in 1995 on 20 patients with advanced HNC. The study focused on the analysis of septic, thrombotic, neurologic, and hemorrhagic complications, and reported a low rate of infectious complications or graft blowout [3]. To prevent postoperative infection, adequate reconstruction of the surgical space was advocated and further supported by several authors [4, 26].

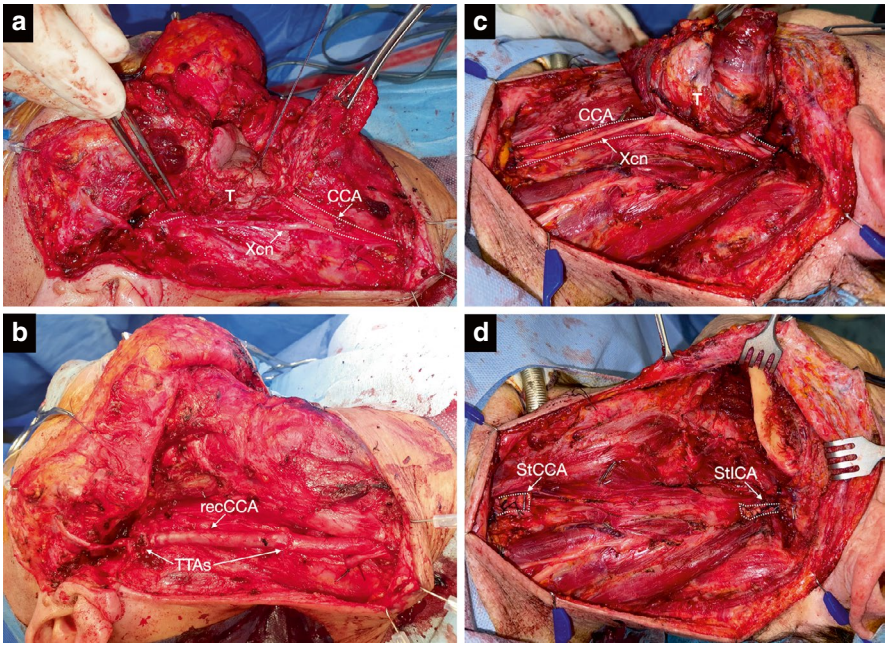


**Fig. 10.4** Common-internal carotid artery resection in patients with regionally recurrent, vascular invading oropharyngeal cancer; (**a**, **b**) resection and primary reconstruction of the common (CCA) and internal carotid artery axis (*dotted lines*) through a greater saphenous vein graft; (**c**, **d**) resection without reconstruction of the common (CCA) and internal carotid artery axis (*dotted lines*). recCCA, reconstructed common-internal carotid artery; StCCA stump of the common carotid artery; StICA stump of the internal carotid artery; T, tumor (nodal metastasis with extranodal extension); TTAs, end-to-end anastomoses; Xcn vagus nerve

Other series demonstrated the feasibility of CA resection and reconstruction with the greater saphenous vein, reporting relatively positive outcomes in terms of survival and disease control in patients affected by very advanced HNC [8] (Fig. 10.4). More recently, Yokoyama et al. proposed to reconstruct the carotid axis by using the superficial femoral vein, which was seen to be as effective as the greater saphenous vein and also to be superior in terms of size, length, and ease of harvesting [11]. Besides venous grafts, in 1998 Sessa et al. published a series of 30 patients with primary, metastatic, or recurrent squamous cell carcinoma (SCC) involving the neck, describing reconstruction of the extracranial carotid axis via a superficial femoral artery graft, achieving favorable results comparable to CA ligation or shaving [30]. Arterial grafts were claimed to be more resistant to infection and superior in terms of long-term patency compared with venous ones, confirming the pioneering observations of Stoney and Wylei who first described the use of arterial grafts in 1970 [32].

When dealing with the cranial base and intracranial segments of the ICA, reconstruction is often unfeasible. An alternative strategy for brain revascularization is represented by bypass surgery, which consists in harvesting a

communication between a donor arterial system and cerebral vascularization through a vascular graft. This surgical procedure represents a fundamental element of the armamentarium required in advanced SBC. Bypass surgical techniques for management of brain pathology do not represent a novelty, with several series dating back to the 1960s. Specifically, in SBC cases several bypass surgery techniques are available, varying in terms of the graft that is employed (i.e. artery vs vein), donor arterial system (i.e. CCA/ICA stump vs external carotid artery) and laterality (i.e. ipsilateral vs contralateral), and timing (i.e. simultaneous to cancer ablation vs staged procedures). However, bypass surgery introduces a complex step in the treatment path of patients affected by SBC, often delaying therapeutic surgery and exposing the patient to substantial risks. A series of 18 SBC patients treated with ICA resection and extracranial-intracranial bypass was published by Kalani et al. [13]. Survival outcomes were unfavorable, with a significant rate of complications, which led the authors to discourage such an aggressive treatment in patients with SBC. The authors reported a mean overall survival of 11.8 months, with cases of surgery-related deaths. Bypass-related and tumor resection-related morbidity rates were 16.7% and 33.3%, respectively. While other series have reported more favorable results [27], adequate selection becomes even more crucial when a bypass surgery is deemed necessary, since the additional risks inherent to this procedure should be clearly counterbalanced by a reasonable chance of tumor control and/or cure.

## *How Accurately Can We Predict Tolerance to Carotid Artery Resection?*

It has been acknowledged that carotid artery ligation without replacement can lead to lethal cerebral infarction, which, according to historical series, ranges from 17% to 40% in unselected patients [33, 34]. Initially conceptualized by Rudolph Matas in 1911, the temporary balloon occlusion (TBO) test was first introduced by Serbinenko in 1974 to preoperatively infer tolerance to permanent vessel occlusion [35]. The advent of TBO, which firstly consisted of neurologic examination for 20–30 min over the occlusion period, has substantially reduced the incidence of postoperative stroke in patients requiring CA resection. However, the first experience with this technique was still flawed by a considerable rate of false negative results, ranging from 15% to 22%, and a TBO-specific complication rate as high as 3–4% [36, 37].

Over time, additional methods have been employed to estimate brain perfusion during TBO, including stump pressure measurements, electroencephalography, evoked potentials, near-infrared spectroscopy, transcranial Doppler ultrasonography, angiographic assessment of the crossflow timing (classified as either synchronous if contralateral cortical veins opacified within 0.5 s or nonsynchronous otherwise), CT (contrast-enhanced, xenon-enhanced), single-photon emission CT, positron emission tomography, and MR perfusion imaging. However, the diversity

of those protocols and their heterogenous ability to predict neurologic adverse events after CA resection make it difficult to draw clear conclusions on the diagnostic performance of this method. A recent meta-analysis by Butterfield et al. included 32 studies conducted in the period 1991–2020 and aimed to investigate the rate of symptomatic ischemic events after a negative TBO [38]. The analysis showed no significant variations in efficacy among the different methods employed to assess brain perfusion under TBO, and resulted in an overall false negative rate of 3.7%. Specifically, the false negative rate was 3.8% with angiography, 2.2% when a CT-based method was adopted, and 5.3% when using 2 or more of the other above-mentioned methods. On one hand, these results did not allow to pick a clear winner among the available diagnostic strategies to test CA resection tolerability. On the other hand, the pooled false negative rate, which was substantially lower than that reported in historical series, should be considered the current performance benchmark of carotid TBO test. Of note, several methods were grouped together in the pooled analysis, thus mandating caution in attributing the false negative rate to poorly represented methods. The authors suggested that TBO monitoring method should be selected based upon the experience of the team, with the intent to minimize risks of complication and invasiveness. Even though these results highlight a low rate of symptomatic ischemic events after a negative TBO, it is clear that the risk of adverse events cannot be entirely nullified, as alongside with the effect of a decreased perfusion pressure there are additional factors that can provoke brain injuries, such as distal embolic or thrombotic event after occlusion or ligation of the CCA/ICA. Moreover, Butterfield et al. reported a pooled TBO-specific complication rate of 0.8%, which is non-negligible even if it was substantially improved compared with the earliest studies on TBO [36, 37].

## *Is the Risk-Benefit Ratio Acceptable?*

When CA sacrifice is planned, a variety of aspects must be pondered through a critical analysis performed in a multidisciplinary setting. Analysis of the therapeutic strategy should be case-by-case and carefully weigh benefits against risk: on one side, prognostic outcomes alongside with the inherent quality of life following surgery should be realistically estimated; on the other hand, possible complications and planned sequelae of treatment should be considered. Counseling should include adequate information about the available evidence and must entail a discussion of therapeutical alternatives that do not include CA resection.

Previous series of CA-resection including surgery for HNC/SBC reported very unsatisfactory outcomes, with 2-year overall survival ranging between 14.3% and 50% [5, 7–9, 12–15, 25, 26]. More recently, series with 2-year overall survival ranging from 60% to almost 90% have been reported [10, 11, 27]. This improvement most likely expresses an increased understanding of very advanced HNC/SBC, which translates into refinement of patient selection. Thus, recognizing prognostic factors that predict an unfavorable outcome is of utmost importance to avoid

unnecessarily aggressive treatments in patients who have little chance of benefitting from surgery. Among all prognosticators of very advanced HNC/SBC, histology is likely the most relevant one. Kalani et al. discussed their poor survival outcomes, commenting that their series was composed by a majority of skull base-invading SCC and highlighting that among 5 patients surviving more than 1 year, only one was affected by SCC [13]. In a series of 10 patients receiving CA resection and reconstruction, 5-year overall survival was <20% versus 100% for SCC and non-SCC malignancies, respectively [11]. While based on a very low level of evidence, one could conclude that very advanced non-SCC cancers, with special reference to those putatively associated with slow and indolent growth, are the best candidates for CA resection-including surgery. In contrast, the indication to such a surgical procedure in patients affected by SCC should be given cautiously, since the risk-benefit balance is unlikely in favor of surgery (Fig. 10.5).

On the other side of the scale, contemporary series reported perioperative mortality, neurologic and non-neurologic morbidity rates of 7–10.0%, 5–20.0%, and 10%, respectively [4, 27, 39]. This proves a substantial improvement compared to the morbidity and mortality profile of the earliest experiences of CA resection-including oncologic surgery, with morbidity and mortality reaching up to 45% and 58%, respectively [3, 4, 8–11, 25, 26, 39, 40]. Overall, SBC are associated with a less favorable morbidity profile compared to HNC, with mortality ranging from 16.0% to 50.0% [12–15, 24] and non-neurologic morbidity from 25.0% to 60.0% [12–14, 24]. Unfortunately, there is a substantial knowledge gap in terms of predictors of severe adverse events following CA resection-including surgery. Thus, selection of patients relies on general concepts that enable gross prediction of increased risk of complications, including age, general conditions, performance status, frailty/vulnerability, and comorbidities. During surgery and in the postoperative period, particular attention should be given to stability of the cardiovascular system and blood count, since arterial hypotension and/or anemia might easily elicit brain ischemia in an otherwise compensated brain circulation. Surprisingly, the preventive use of brain revascularization techniques has not been associated with an improvement in terms of morbidity profile [41].

## *Are There Therapeutic Alternatives?*

Although in selected patients a radical surgical approach offers the highest chances of cure, alternative therapeutic options may be offered to HNC and SBC patients with CCA/ICA involvement. These include curative photon or particle RT alone, concurrent chemoradiation (CRT), or palliation [42–45]. Roughly one-third of HNC are addressed to these alternative approaches, being defined as “unresectable” owing to the impossibility to achieve negative margins, or due to the unfavorable balance between potential oncological benefits and risks related to surgery. However, very little is known in terms of oncological outcomes as data on non-surgically treated patients are scant. In a cohort of 23 patients with HNC invading the carotid

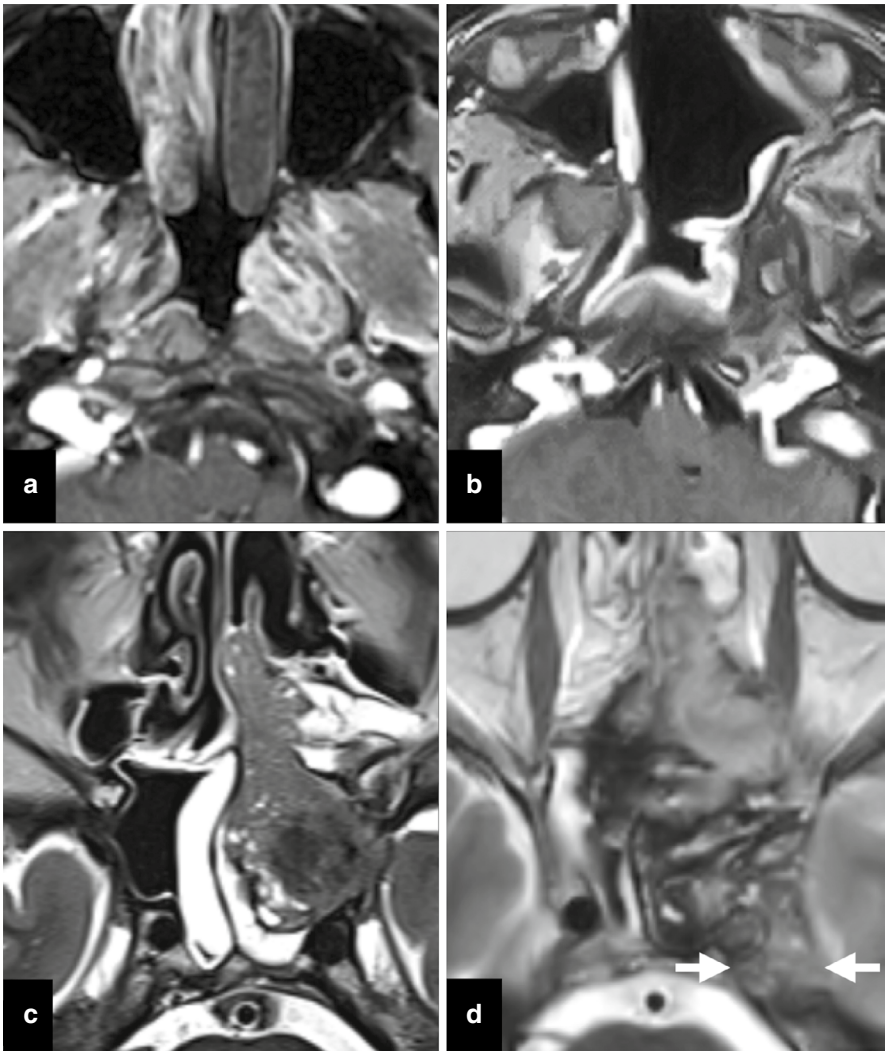


**Fig. 10.5** Opposite outcomes in patients who underwent carotid artery-including ablations; (**a**, **b**) salivary polymorphous adenocarcinoma of the nasopharynx treated with internal carotid artery-including endoscopic resection and adjuvant radiotherapy; the right image shows disease-free imaging 4 years after treatment completion; (**c**, **d**) recurrent nasopharyngeal carcinoma treated with internal carotid artery-including endoscopic resection; the patient denied adjuvant re-irradiation and remained disease-free for 18 months, then developing local recurrence into the Meckel's cave (*arrows*) and orbital apex

artery, the group treated with surgery showed a median survival time of 16.5 months, whereas the group receiving chemoradiation or palliative treatment achieved a median survival of 11.5 and 3 months, respectively [16]. A subsequent study reviewed a larger cohort of 44 patients affected by SCC with CA involvement,

including both primary and recurrent HNC [46]; 35 patients were treated with curative intent, including upfront CA resection-including surgery, upfront CA-sparing surgery, or definitive chemoradiation. The different strategies adopted in this subcohort were not significantly different in terms of survival ($p = 0.47$). Another study on a series of 67 patients affected by resectable CA-involving N3bM0 SCC of the head and neck demonstrated substantially different outcomes (*i.e.* 1-year OS) when comparing treatment modalities in primary or salvage setting [47]. Patients were treated with either definitive RT/CRT, upfront surgery, or systemic therapy. In the primary setting, RT/CRT was associated with significantly higher 1-year OS compared with upfront surgery (73% vs 40%, respectively; $p < 0.001$). However, surgery provided significantly better 1-year OS than systemic treatment in the salvage setting (40% vs 14%, respectively; $p = 0.024$). Of note, distant metastasis occurred in more than 50% of cases, irrespective of treatment. Thus, when one considers CA-invading primary SCC of the head and neck, the available evidence suggests that non-surgical treatment confers the best survival, with CA resection-including surgery to be considered in the salvage setting.

However, CA-sparing treatments (i.e., CA-sparing surgery and non-surgical treatments) can also lead to life-threatening complications, which are mostly represented by CB, which was associated with a 76% lethality rate in previously irradiated patients [48, 49]. Different factors have been identified that increase the risk of CB. In a recent series of 1072 patients, Jacobi et al. found that increasing T stage was associated with a progressive raise of the cumulative incidence of CB. Several series have demonstrated the prominent role of re-irradiation in determining an increased risk of late CB in HNC patients [50]. Garg et al. systematically evaluated the dose administered to the CA, highlighting that patients receiving a cumulative dose of 120 Gy or more achieve the risk of blowing out within 1 year as high as 25% [51]. All these aspects concur in determining a challenge for the radiation oncologist, who needs to decide whether performing a suboptimal treatment decreasing the dose close to the CA or taking the risk of triggering CB. Unfortunately, to date no data are available on the utility of CA stenting/occlusion before CRT/RT.

Regarding CA-sparing surgery, Ozer et al. reported on 64 patients affected by CA-abutting HNC treated with subadvential dissection without resection of the artery. CB was the most relevant complication, presenting with an incidence of 3–4.5% [8]. Markiewicz et al. investigated the role of preoperative CA stenting. They described 5 cases treated with preoperative stenting and subsequent tumor ablation including the excision of the adventitia from the stent. No intraoperative complications were registered. One patient experienced a postoperative stent occlusion with subsequent stroke [52]. The main drawback of preoperative stenting is the need for double antiplatelet therapy, which increases the risk of intra- and post-operative bleeding.

Overall, while CA resection-including surgery is not free of complications, it allows to avoid two very unfavorable scenarios represented by positive/close margin on the carotid axis and CB. Accurate case-by-case analysis is paramount to identify cases for which an apparently less aggressive CA-sparing approach might expose the patient to an increased risk not only of recurrence, but also of life-threatening complications.

## Conclusions

CCA/ICA resection can be indicated in very selected patients affected by HNC/SBC. Case selection is of utmost importance and should take into account tumor location and extension, primary *vs.* recurrent setting, histology, patient's motivation, and factors that substantially affect the risk of perioperative complications. Overall, non-SCC cancers with slow growth and limited aggressiveness are the best candidates for CA resection-including surgery. SCC of the head and neck with CA-involvement should be considered cautiously for such an aggressive surgery, since non-surgical strategies have demonstrated similar or even better results in the primary setting. Multidisciplinary management is essential to select patients and achieve an optimized patient-centered approach [53]. The underlying risk-benefit balance needs a comprehensive and thoughtful approach to achieve favorable oncologic outcomes while not exposing the patient to unreasonable morbidity.

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# Chapter 11
# Improving Quality of Reconstructive Surgery in Head and Neck Cancer


**Prady Naredla, Prav Praveen, and Sat Parmar**

## Introduction

Head and neck cancer presents formidable challenges due to the complex anatomy of the region but also due to functional considerations such as speech and swallowing. Reconstructive surgery serves as a cornerstone in restoring both form and function. This chapter delves into pivotal advancements geared towards improving the quality of reconstructive surgery in head and neck cancer, highlighting modern innovative technology using computer assisted planning.

## Aims of Reconstruction

Central to head and neck cancer reconstruction is the restoration of aesthetic harmony, vital for bolstering patients' self-esteem and social interactions. Facial disfigurement stemming from surgery will impact on emotional health and interpersonal relationships. Hence, meticulous reconstruction aimed at achieving natural outcomes is indispensable for nurturing patients' confidence and overall contentment with their appearance. Beyond aesthetics, reconstruction endeavours to restore functional integrity to the head and neck region, pivotal for maintaining essential physiological processes. Key functional considerations include:

**Speech**: preservation or restoration of articulate speech is paramount for effective communication. Reconstruction of the oral cavity and pharynx should prioritise maintaining articulatory mechanisms and airflow dynamics to facilitate clear and intelligible speech.

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**Mastication**: restoring the ability to chew and swallow food comfortably is essential for adequate nutrition and overall well-being. Reconstruction efforts should focus on reinstating normal occlusion, muscle function, and oral sensation to optimise masticatory efficiency and prevent dietary limitations**.**

**Swallowing**: effective swallowing function is crucial to prevent complications and ensure proper nutrition. Reconstruction of the pharynx and larynx should emphasise preserving or restoring swallowing mechanisms to facilitate safe and efficient oral intake.

**Quality of Life**: ultimately, the overarching goal of high-quality reconstruction is to enhance patients' overall quality of life, encompassing physical, emotional, and social dimensions. Successful reconstruction minimises physical impairments, boosts self-esteem, fosters social integration, and facilitates the resumption of daily activities [1].

Patients benefitting from successful reconstruction report enhanced psychosocial adjustment, reduced psychological distress, and heightened satisfaction with post-treatment outcomes. This translates into a tangible improvement in overall well-being and a more positive outlook on life. Successful reconstruction also allows the patient to return to work and thus providing economic benefit to his family and the country.

## Strategic Assessment of Surgical Defects

Effective reconstruction following head and neck cancer surgery hinges upon meticulous assessment of surgical defects, encompassing a multifaceted evaluation of structural loss, defect dimensions, surrounding tissue viability, and patient-specific factors. This section underscores the pivotal role of comprehensive evaluation in informing reconstructive strategies and optimising patient outcomes. The following elements are of importance:

**Structural Loss**: identification of type and quantity of lost structures post-cancer resection should be assessed in terms of soft tissue, bone, and supportive elements like cartilage or nerves. This will guide reconstructive approaches, ensuring both functional rehabilitation and aesthetic restoration.

**Defect Dimensions**: evaluation of defect dimensions, encompassing length, width, and depth, serves as a compass for selecting appropriate reconstructive techniques and determining tissue or graft requirements for achieving optimal coverage and support.

**Tissue Vascularity**: assessment of surgical bed vascularity to assess what type of reconstruction is required. This will also determine the likelihood of wound break down.

**Radiation Influence**: recognition of radiation therapy effects on tissue quality and vascularity is crucial. Radiation-induced alterations like fibrosis or compromised vascularity necessitate nuanced adjustments in reconstructive strategies to

mitigate risks and optimise outcomes. Radiation may also make vessel access for free tissue transfer difficult.

**Scar Tissue Impact**: evaluation of surgical scarring's extent and impact guides flap design, tissue mobility considerations, and wound closure strategies, aiming to alleviate contracture and optimise functional outcomes.

**Tobacco-Related Considerations**: acknowledgment of tobacco's influence on tissue vascularity and wound healing underscores the importance of smoking cessation interventions preoperatively to minimise complications like flap failure or infection.

**Integration with Radiation Therapy**: consideration of adjuvant radiation therapy implications on tissue healing and long-term outcomes is vital. Seamless coordination with radiation oncologists ensures harmonious integration of reconstructive plans with adjunct treatment strategies.

**Infection Risk Assessment**: evaluation of infection risks, predicated on prior surgical interventions, compromised vascularity, or immunocompromised states, underscores the importance of prophylactic measures and stringent wound care protocols.

**Patient Centric approach**: integration of patient-specific factors like age, medical history, nutritional status, and functional requisites tailors reconstructive plans to individual needs, optimising outcomes while mitigating risks and complications.

## Advances in Three-Dimensional Bony Reconstruction Techniques

Three-dimensional bony reconstruction techniques have ushered in a new era in maxillofacial surgery, providing precise solutions for restoring skeletal integrity post-tumour resection or traumatic injuries. This section explores various methods of three-dimensional (3D) bony reconstruction, emphasising pre-bent plates using 3D models and virtual planning, along with their implications for surgical practice [2, 3]. It is based on techniques routinely used in Birmingham.

There are a number of essential steps required to achieve success in 3D planning. Firstly, accurate digital design is needed. A recent computed tomography (CT) scan in DICOM (Digital Imaging and Communications in Medicine) format with thin slices should be obtained. If planning will be used for both the ablative and reconstructive components, then a scan of the proposed donor bone is also necessary. Often the imaging will need to be 'cleaned-up' to remove artifacts. In the oral cavity, dental restorations may create significant scatter artifacts and software systems may be needed to remove this. Accurate planning is the next step to undertake. This should be multi-disciplinary in nature with the surgeon, technician and possibly restorative dentist involved. In modern practice most propriety companies will undertake these sessions virtually. If design and manufacture is undertaken 'in-house' then these sessions may be more frequent and also allow for rapid feedback and modification if required. Planning at this stage will consider the resection and

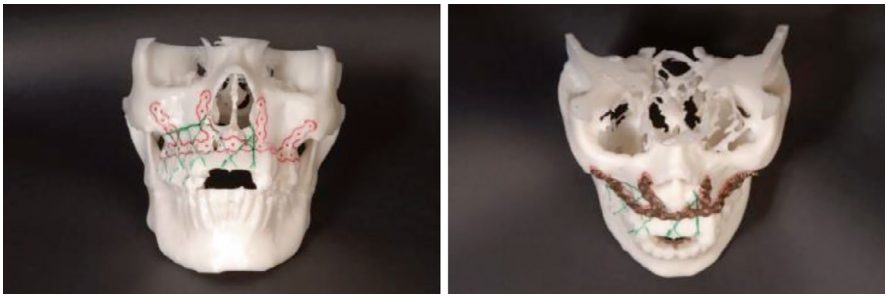

**Fig. 11.1** 3D model printed in house, with resection margins and pre-bent plate

surgical margins to be obtained as well as any involved or adjacent structures. Other issues that may need to be considered include how best to register cutting guides and the position of screws with regard to potential implant rehabilitation (Fig. 11.1).

When 3D planning was initially developed, its use was often limited to complex reconstruction cases. As recent years have seen a reduction in costs and manufacturing time, some units would now consider 3D planning for the majority, if not all, of their bony reconstruction cases. The process remains most beneficial in complex defects of the maxilla and mandible, such as those requiring a large amount of soft and hard tissue resection and/or multiple osteotomies of the donor flap [6]. The process is also very beneficial when dental implant rehabilitation is planned. Short span mandibular and low-level maxillary defects may not require 3D planning but may still benefit from the improved accuracy possible in both resection and reconstruction, as discussed below.

## Pre-bent Plates Using 3D Models

This technique involves bending standard plates to 3D models which have been modified to match the contour of the defect based on imaging data. These plates facilitate precise intraoperative placement, enhancing surgical accuracy.

## Advantages of Virtual Planning and 3D Bone Reconstruction

**Cost-Effectiveness**: Virtual planning and pre-bent plates can reduce costs by minimising intraoperative time and potential complications, making them economically viable options [4, 6, 7].

**Complex Configuration**: three-dimensional reconstruction enables the fabrication of intricate plate designs, optimising stability and biomechanical support for improved outcomes.

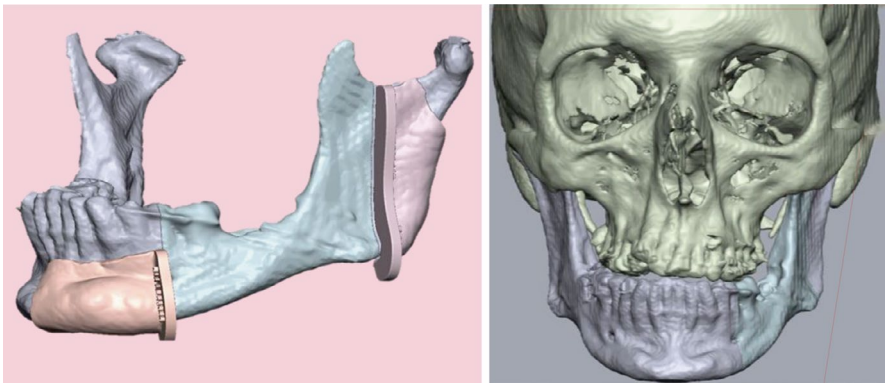

**Fig. 11.2** Virtual surgical planning with cutting guides

**Enhanced Surgical Results**: precise pre-operative planning enhances surgical accuracy, leading to improved outcomes and heightened patient satisfaction, fostering better postoperative recovery.

**Virtual Planning—Industry**: Industry-based virtual planning services offer access to specialised expertise and resources for complex reconstructions. Surgeons collaborate with engineers and technicians to develop customised solutions, leveraging industry partnerships for enhanced precision.

Industry-based virtual planning offers predictable and accurate reconstructions, decreased operating times, and patient-specific solutions tailored to individual anatomy.

However, it may be associated with higher costs, longer lead times, and concerns regarding plate removal or fracture [10] (Fig. 11.2).

## Virtual Planning: In-House

In-house virtual planning employs advanced imaging software to simulate the surgical procedure and design patient-specific implants or cutting guides. Surgeons can visualise the defect, plan the reconstruction, and fabricate custom solutions tailored to individual patient anatomy.

In-house 3D planning contributes to enhanced outcomes in both tumour resection and subsequent reconstruction, leading to reduced ischaemic time, superior cosmetics, improved function, and easier placement of implants. Streamlined workflow facilitated by in-house 3D planning leads to reduced operating times, ultimately lowering overall costs. Efficient pre-operative planning optimises resource utilisation and minimises intraoperative complications. In-house 3D planning fosters innovation within surgical teams, encouraging the development of novel techniques and solutions. Additionally, it provides collaboration opportunities with

industry partners, driving commercial development in maxillofacial reconstruction. By eliminating the need for outsourcing, in-house 3D planning shortens the time required for the fabrication of surgical guides and patient-specific implants, accelerating the overall treatment timelines [8].

In-house virtual planning offers predictable and accurate reconstructions with customised patient-specific plates, ensuring optimal fit and alignment for improved surgical outcomes.

Despite its advantages, in-house virtual planning still relies on the availability of 3D models and requires additional steps such as plate bending and sterilisation, introducing potential delays and workflow complexity.

## Disadvantages of In-House Virtual Planning and 3D Bone Reconstruction

**Cost Implications**: initial investment in technology and software may be substantial, particularly for in-house setups, potentially limiting accessibility.

**Manpower Requirements**: skilled personnel are essential for virtual planning and fabrication of patient-specific implants, adding to overall costs and resource demands.

**Cutting guides**: Cutting guides may guide resection and thus reducing close or positive margins of tumour resections. Cutting guides also serve as indispensable aids for achieving optimal flap inset and alignment, minimising intraoperative adjustments and surgical duration. By replicating defect dimensions and contours, these guides ensure seamless integration of reconstructed tissue with surrounding anatomy, thereby enhancing both functional and aesthetic outcomes. Collaborative efforts between surgeons and biomedical engineers drive the design and fabrication of cutting guides, harnessing advanced computer-aided design (CAD) and 3D printing technologies. By translating imaging data into bespoke guides, surgeons can strategise and execute bony reconstruction with unparalleled accuracy and assurance [9, 10, 13].

The integration of cutting guides marks a significant leap forward in head and neck cancer surgery, delivering heightened precision, efficiency, and patient outcomes in bony tissue reconstruction. Continued technological evolution promises further enhancements in guide design and utilisation, further elevating the standard of care for reconstructive surgery patients.

## Resection

The primary aim of ablative head and neck surgery is to remove any lesion with adequate histological margins. In oncological practice this will generally be a 1 cm surgical margin. Where possible, aesthetic units and vital structures should be

preserved although this should not take precedence over oncological clearance. In the mandible, if resection margins are close to the condyle, a decision must be made on whether to preserve this segment or to incorporate it into the resection. 3D planning allows for the creation of specific cutting guides to accurately achieve the planned resection intraoperatively [11, 12]. The guides and planned resection margins may also allow for the potential of interval tumour growth between the planning stage and the actual time of surgical resection. After the lesion is exposed intra-operatively, the custom guides are seated on the mandible or maxilla. The guides should be constructed so as to seat passively and directly adjacent to the bone and may have special locators to assist in this. The guides may then also be secured with mono-cortical screws. The bony resection is then undertaken without concern for stabilising the bony remnants. If planned, the guides may also contain guide holes to be drilled to allow later screw placement for securing the reconstruction plates [15–17].

## Mandibular Reconstruction

Composite free flap reconstruction is now the gold standard in mandibular reconstruction. When planning the reconstruction, the height and vertical position of the donor bone is a key consideration. A lower position along the mandibular border maximises form while a higher position near the alveolus will allow for easier implant placement and dental rehabilitation. Once the bony position is confirmed, a 3D model of the planned reconstruction can be printed and a plate contoured to this. The plate length is determined so as to ensure there are three screws on either side of the resection. Intra-operatively, the pre-bent plate is seated onto the native mandible following resection using temporary localisation guides soldered to the plate. This ensures an accurate fit prior to placement on the donor bone. If the cutting guides did not contain guide holes, then the plate can be used to pre-drill screws holes at this time. The plate is then removed and the localisation guides removed. The plate is secured onto the donor bone with mono-cortical screws so as to avoid damage to the vascular pedicle on the deep aspect of the bone. Usually, two screws are placed per bony segment. The plate and flap reconstruction is then inset into the defect and secured using the pre-drilled holes and bi-cortical screws (Figs. 11.3 and 11.4).

There are a number of bony flaps that may be used for mandibular reconstruction. A number of considerations such as the quantity and quality of hard and soft tissue required, if dental implant placement is planned and the pedicle length required will help guide selection. The fibula free flap remains the work-horse flap of mandibular reconstruction for a number of reasons. Its lower limb location makes it easily accessible and permits two teams operating thus shortening operative time. It is straight-forward to raise, provides a significant length of bone and results in low levels of donor site morbidity. Importantly, it also allows for 3D planning and the construction of specific cutting guides. Although the bone stock may be limited in vertical and cross-sectional dimensions, dental implant rehabilitation is usually

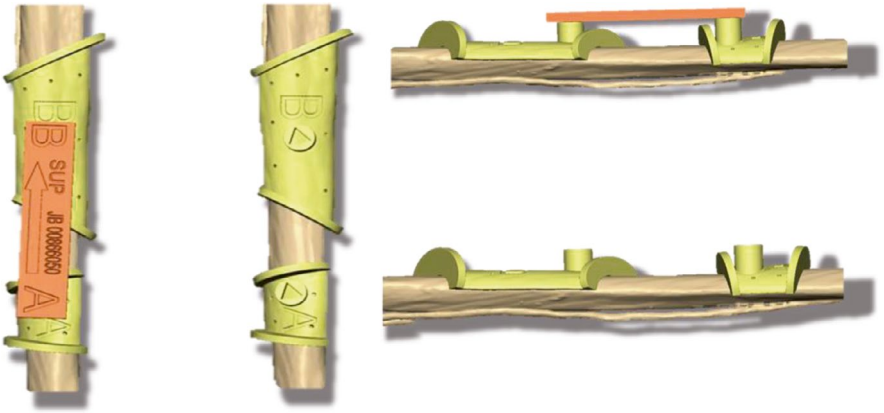


**Fig. 11.3** Birmingham Fibula cutting guide

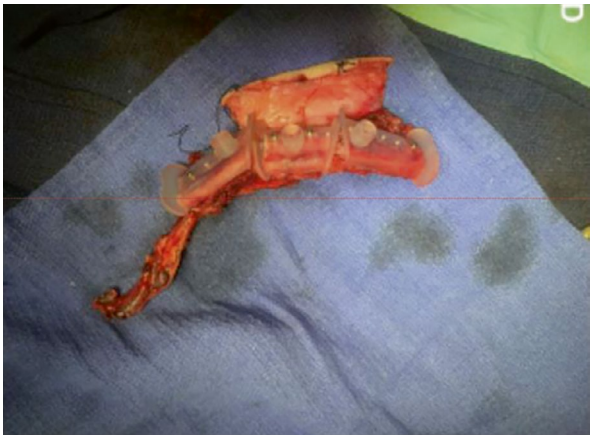

**Fig. 11.4** Osteotomised fibula free flap

possible. Other flaps that may be considered include the deep circumflexiliac artery (DCIA) flap, the composite scapula flap and the composite radial flap. All of these flaps have their own individual advantages and disadvantages with regard to mandibular reconstruction.

The DCIA flap provides good bone stock although the length of vascular pedicle is often a concern. A two-team approach is also permitted with this flap and although it may be raised as an osteo-cutaneous composite flap a myo-osseous flap utilising the internal oblique muscle. Donor site morbidity is a concern with this flap and meticulous closure is required to minimise the risk of hernias. The composite scapula flap is often considered when large volumes of soft tissue is required in addition to bony reconstruction. The flap, based on the subscapular arterial system, may also be raised in a chimeric fashion to provide two or more reconstructive components. The composite radial free flap is less commonly used in mandibular reconstruction due to the limited amount of bone stock available and the potential for donor site morbidity.

## Maxillary Reconstruction

### *Objectives*

**Closure of Palatal Defect**: foremost among the goals of maxillary reconstruction is the attainment of complete closure of the palatal defect ensuing from tumour excision. This closure reinstates oral integrity, fosters speech articulation, and forestalls nasal cavity ingress of food and fluids.

**Speech and Swallowing Optimisation**: maxillary reconstruction endeavours to optimise speech articulation and swallowing functionality, often compromised by residual defect. By restoring normative oral anatomy and function, clear speech and safe swallowing are facilitated.

**Maintenance of Dentition**: preservation or restoration of dentition stands pivotal in upholding occlusal stability and facilitating effective mastication. Dental rehabilitation interventions, encompassing dental implant placement or prosthetic fittings, bolster oral function and enhance quality of life.

**Effective Mastication**: maxillary reconstruction strives to reinstate masticatory function by reconstructing the hard palate and its adjunctive structures. Adequate masticatory function fosters efficient food chewing, ensuring nutritional adequacy and fostering overall well-being.

**Aesthetic Restoration**: aesthetic considerations wield substantial significance in maxillary reconstruction, aiming to rejuvenate facial symmetry, contour, and harmony. The reconstruction endeavors to yield natural outcomes, seamlessly melding with the surrounding facial topography.

**Cheek Prominence and Facial Contour Restoration**: incorporating techniques like midface advancement and soft tissue augmentation, maxillary reconstruction endeavors to revive cheek prominence and facial contour, thereby augmenting facial aesthetics and fostering a youthful appearance.

**Orbital Support**: maxillary reconstruction aspires to furnish adequate orbital support, thereby restoring orbital anatomy and precluding conditions such as enophthalmos and orbital dystopia. This endeavor safeguards visual function and upholds facial symmetry.

Maxillary reconstruction in the realm of head and neck cancer surgery harbours manifold objectives, spanning functional restitution, aesthetic enhancement, and the amelioration of overall quality of life. By addressing the diverse needs of patients, reconstructive initiatives strive to optimise outcomes and facilitate the seamless rehabilitation of individuals afflicted by maxillary defects [14]. Interdisciplinary collaboration among surgeons, prosthodontists, speech therapists, and allied team members assumes paramount importance in realising comprehensive maxillary reconstruction and fortifying patient outcomes.

The Brown classification system offers a straightforward way to classify maxillary defects based on the vertical and horizontal components. Class I defects are confined to the maxillary alveolus without creating an oro-antral communication.

Class II defects extend vertically to create such a communication but do not involve the orbit. Class III defects involve the orbit with retention of orbital contents, while class IV defects require orbital exenteration or enucleation. Class V and VI defects encompass orbitomaxillary and nasomaxillary regions, respectively, each presenting unique challenges for reconstruction.

Similar to mandibular reconstruction, 3D planning plays a vital role in maxillary reconstruction. This typically involves creating printed models to facilitate the fabrication of cutting guides and pre-bent plates tailored to the planned reconstruction. Such advanced planning ensures precision and efficiency during surgery.

In cases of low-level defects (class I and some class II), soft tissue reconstruction and the use of prosthetic obturators may suffice. However, for larger or anterior class II defects, a bone strut is often required. Flaps such as the fibula or scapula flap may be suitable options in these cases.

Class III defects present significant challenges due to the loss of alveolar bone, the orbital floor, and cheek support. While no single flap can address all these issues, the DCIA flap with a custom orbital reconstruction plate is often considered the optimal choice. This flap can be harvested and contoured to reconstruct the lost maxillary buttresses, with 3D planning facilitating the process.

Class V (orbitomaxillary) and VI (nasomaxillary) defects are less common and require tailored approaches. In class IV cases, the primary goal is to create a foundation for future prosthetic rehabilitation without reconstructing the orbital floor. Flaps such as the DCIA or scapula flap can provide adequate muscle bulk to fill the orbital defect [5].

Reconstruction of class V defects typically requires minimal bone, making the composite radial forearm free flap a viable option. Each reconstruction approach should be carefully tailored to address the specific challenges posed by the defect, ensuring optimal functional and aesthetic outcomes for the patient.

## Soft Tissue Reconstruction

Soft tissue reconstruction stands as a pivotal aspect of post-surgical recovery in head and neck cancer patients, addressing both functional rehabilitation and aesthetic restoration.

Perforator flaps represent a pioneering technique in soft tissue reconstruction for head and neck cancer surgery, capitalising on vascular anatomy principles. These flaps offer the transfer of well-vascularised tissue while minimising donor site complications. By preserving adjacent tissues and reducing the reliance on distant flaps, perforator flaps present multifaceted benefits for functional and aesthetic outcomes. Meticulous preoperative planning underpins the success of perforator flap reconstruction, involving the identification of suitable vessels and tailored flap design based on defect characteristics. With advances in imaging modalities like Doppler ultrasound and CT angiography, surgeons can accurately map perforator vessels, facilitating precise tissue transfer.

The versatility of perforator flaps allows for the reconstruction of diverse soft tissue deficits, encompassing skin, muscle, and composite tissue losses. Prominent examples include the anterolateral thigh flap and deep inferior epigastric perforator flap. These flaps offer ample tissue bulk and vascularity, enabling intricate defect reconstruction while minimising donor site morbidity.The adoption of perforator flaps has redefined soft tissue reconstruction paradigms in head and neck cancer surgery, ushering in superior functional outcomes, refined aesthetic results, and decreased postoperative complications compared to conventional flap techniques (Fig. 11.5).

In tandem with advancements in flap selection and harvesting, the integration of cutting guides streamlines the soft tissue reconstruction process in head and neck cancer surgery. These patient-specific guides, derived from preoperative imaging, facilitate meticulous shaping and trimming of flaps to precisely match defect contours.

While techniques for bony reconstruction have evolved with patient-specific planning and 3D printing, soft tissue reconstruction hasn't seen similar advancements. Koumoullis et al. in 2020, described the "PANSOFOS" flap, a personalized approach to soft tissue head and neck reconstruction. Following the IDEAL framework for surgical innovations, they presented a patient with oral cancer undergoing tongue reconstruction post-hemiglossectomy. Utilising staging scans, 3D printing, and software, they created a soft silicon resection guide and flap harvesting guide. The 3D guide aided in designing the flap's perimeter, and a negative silicone mould controlled its bulk. The procedure was successful with excellent oncological, cosmetic, and functional outcomes. This report demonstrates the feasibility of patient-specific 3D planning in soft tissue reconstruction [18].

Restoring the natural hue of the skin stands as a cornerstone in achieving post-reconstruction aesthetic coherence and patient satisfaction. Preservation of skin

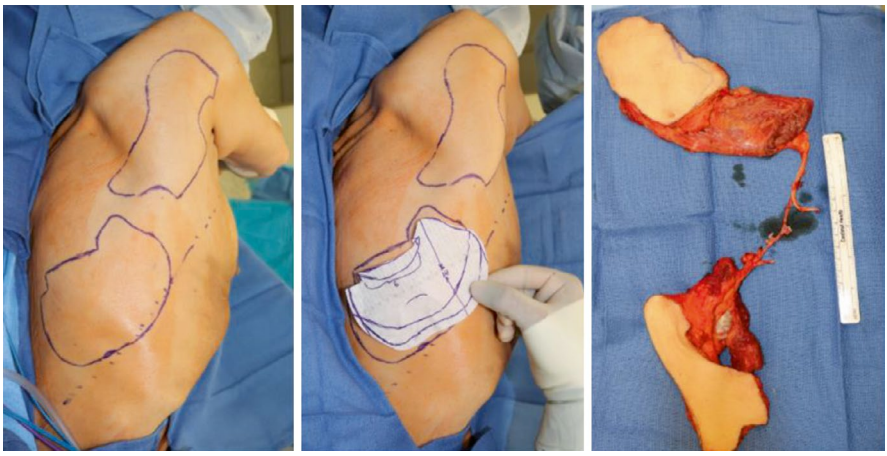

**Fig. 11.5** Chimeric free flap based on sub scapula system

volume and contouring ensures a rejuvenated, symmetrical appearance, thereby elevating aesthetic outcomes and augmenting patient well-being.

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# Chapter 12
# Innovation in Radiotherapy: FLASH Therapy

**Jean Bourhis, Edouard Romano, Olivier Gaide, Veljko Grilj, Jean-Francois Germond, Till Boelhen, Raphael Moeckli, and Remy Kinj**

## FLASH Effect and Its Clinical Translation

FLASH therapy (or FLASH radiotherapy; FLASH RT) is a new and promising form of radiotherapy delivering radiation at a very high dose rate, in milliseconds instead of minutes as in conventional RT (CONV RT). By doing so, a remarquable sparing of normal tissues is consistently observed when comparing FLASH RT versus CONV RT [1–8]. This is illustrated in Fig. 12.1 [7], showing a major sparing with FLASH of late necrosis, 9 months after skin irradiation in pig. This sparing effect on normal tissues of ultra high (FLASH) dose rate was recognized already in the 1970s and 1980s, but at that time the effect on tumors was not studied [1]. About 30 years later, a striking observation came from the seminal publication of Favaudon et al. in 2014 [8], who showed not only much less radiation toxicity on normal healthy tissues with FLASH RT versus CONV RT, but in contrast, the tumor response to FLASH RT and CONV RT was identical [3, 8]. This so-called "FLASH effect" was found to be robust and reproducible in numerous experimental settings of normal tissues and tumors [1–8]. Indeed FLASH sparing effect on normal tissues has been observed with X-rays, electrons, protons, carbon ions beams [1, 6, 11, 17] as well as in several animal species (pig, cat, mouse, zebra fish) [1–8, 13, 14], in most types of tissues in mice while no sparing effects were observed in various tumor types [1].

Based on this strong rationale, clinical translation of FLASH therapy has started both with low energy electrons for superficial cutaneous cancers and with protons for deeper seated tumors. A first treatment of a patient with FLASH therapy was

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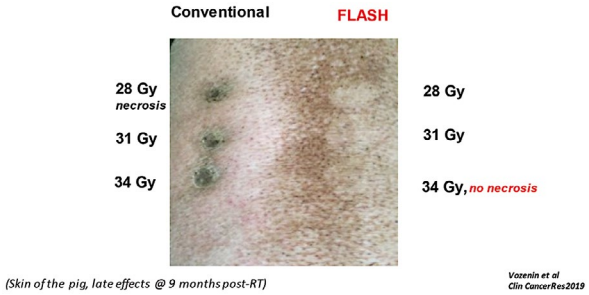


**Fig. 12.1** Illustration of the FLASH effect

performed at CHUV (Lausanne University Hospital) in 2018, showing feasibility, safety and efficacy of delivering 15 Gy in 90 ms with a 5.4 MeV electron beam for a patient with cutaneous lymphoma [9].

The effect of FLASH therapy has a few characteristics that need to be emphasized, to understand how it could be used clinically in an optimal way.

The main parameter to consider for clinical use of FLASH RT is the dose rate. Indeed, an overall delivery time below 100–200 ms is generally needed for obtaining a reproducible FLASH sparing effect on normal tissues [9, 13].

Among other important characteristics, the magnitude of the advantage of FLASH RTcompared to CONV RT appears higher when large doses per fraction are used, i.e. above 20 Gy [2]. This would in turn imply that a maximal differential effect could be obtained with this type of doses (or even with higher doses), suggesting for instance that FLASH RT could be used as a boost for large tumors. However, interestingly, this more pronounced effect with a large dose per fraction does not mean that the FLASH sparing effect is not observed at lower doses. Indeed a first pre-clinical study on fractionation suggested that repeated FLASH fractionated low doses ($10 \times 3$ Gy) also induce a remarkable sparing of mouse brain [12] when compared to the same fractionated CONV RT regimen. This observation is opening the opportunity to enlarge the use of hypofractionated RT in large irradiation fields and prompted to initiate clinical studies testing fractionated FLASH RT versus fractionated CONV RT in randomized clinical trials, which is ongoing for cutaneous cancers [18].

Another intriguying characteristic of FLASH RT is that it appears to be very effective in hypoxic tumors, or at least there is no difference regarding the effect of FLASH RT under oxic or hypoxic conditions which is never observed with CONV RT (constantly less effective in hypoxic tumors). This is a very unique feature suggesting that the differential effect between tumors and normal tissues could be even higher in the most hypoxic tumors and hence for the most radioresistant ones [15, 16].

Two ongoing clinical trials are currently performed at CHUV (Lausanne University Hospital, Switzerland) using low energy electrons for cutaneous tumors, namely IMPULSE (NCT 04986696) which is a dose-escalation phase I trial in metastatic melanoma and LANCE (NCT 05724875) which is a randomized phase III trial comparing FLASH RT and CONV RT for squamous cell and basal cell

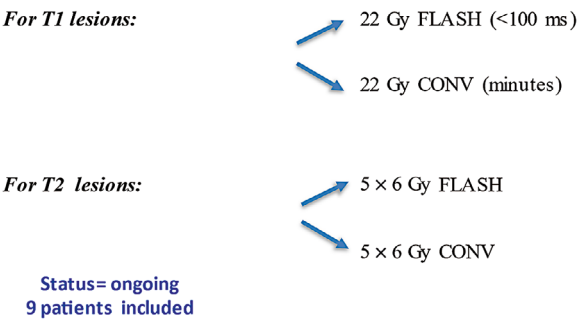


**Fig. 12.2** Lance ongoing randomized trial of FLASH versus CONV for skin basal and squamous cell cancers [18]

carcinomas, using a single dose for T1 and a fractionated dose for T2 tumours [18] (Fig. 12.2).

Achieving FLASH dose rates in large and deep seated tumors, while maintaining high conformity is the next step for clinical translation but also a huge technical challenge. At the moment it appears feasible for clinical translation essentially with proton beams or with VHEE (Very High Energy Electron) [10]. In this context, clinical translation of FLASH therapy has just started for deep seated tumors with proton therapy at UPenn University hospital, initially testing palliative FLASH doses (FAST 01 and FAST 02 clinical trials). In parallel, a FLASH-VHEE project is ongoing at CHUV in collaboration with CERN and the THERYQ company [19] and expected to be ready for treating patients in 2027. FLASH RT will be first tested for unmet clinical needs such as noncurable and/or nonresectable cancers for the which FLASH RT will allow to increase markedly the dose on tumors without increasing side effects. On the long term, FLASH RT may also have other potential advantages, allowing to use less fractions along with an extremely fast treatment delivery time that should ultimately improve patient comfort and reduce waiting time for radiotherapy. In addition, the quasi instantaneous delivery time will allow to suppress the complexity of tumor tracking for tumors that move with respiration.

The biological mechanisms involved in FLASH are not really understood, and one of the most striking differences between FLASH RT and CONV RT is the reduced lipid peroxidation observed with FLASH irradiations, although the biological meaning of this observation is unclear. Another biological feature is that FLASH RT has some level of dependency to the tissue oxygenation since it was shown that hyper-oxygenation in animals could markedly reduce the FLASH sparing effect of normal tissues [5].

## Conclusions

FLASH RT, which delivers radiation dose in milliseconds instead of minutes, appears much less toxic on normal tissues and more efficient in hypoxic tumors when compared to CONV RT. This effect was shown to be robust and reproducible

with different types of beams and across several species as well as in various types of normal tissues and tumors.

FLASH appears to be a new and promising tool for increasing the differential effect between tumors and normal tissues that justifies its ongoing clinical translation and is opening new avenues in radiation oncology and oncology.

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# Chapter 13
# Hypoxia in Head and Neck Cancer: Current Relevance

**Olivier Feron**

## Introduction

Head and neck squamous cell carcinoma (HNSCC) is a serious, genetically complex disease that primarily originates from the mucosal epithelium in the oral cavity, pharynx, and larynx. Currently, there is no screening test effective for HNSCC, and most patients are often diagnosed at late stages, with loco-regionally advanced or metastatic diseases that require multimodal treatments [1]. Along with surgery, radiotherapy (RT) represents the standard-of-care local treatment for HNSCC [2, 3]. Indeed, RT plays a versatile and crucial role in the management of HNSCC with applications ranging from definitive treatment (when surgery is not the primary treatment option or is not feasible) to adjuvant therapy (after surgery), used alone or in combination with chemotherapy (known as chemoradiation, mainly to reduce the risk of recurrence) [2, 3]. RT effectiveness relies mainly on its ability to induce lethal DNA double-strand breaks (DSBs) and thus on the possibility to take advantage of a deficient DNA damage response (DDR) resulting from genetic alterations or upon co-treatment with drugs impacting on DNA damage sensing and repair [4].

A prominent feature contributing to radioresistance (but also disease progression) in HNSCC patients is hypoxia. Tumor hypoxia results from an imbalance between oxygen consumption and limited oxygen delivery due to the spatio-temporal disorganization of the tumor vasculature [5, 6] (Fig. 13.1a). Hypoxia impairs the efficacy of RT (Fig. 13.1b), primarily because of a deficit in the stabilization of radiation-induced DNA damage by oxygen (which normally converts free radical-induced DNA damage into more permanent forms that are harder to repair)

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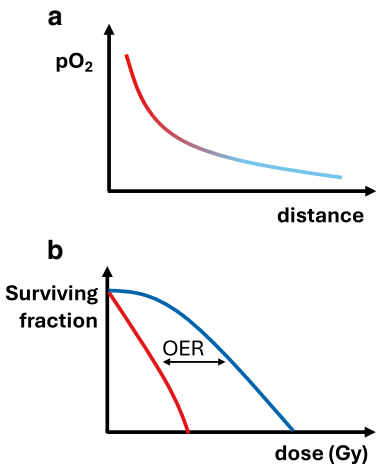


**Fig. 13.1** The basics of tumor hypoxia and the oxygen effect. (**a**) Hypoxia, or low oxygen levels, tends to escalate as one moves farther away from tumor blood vessels. (**b**) OER, or Oxygen Enhancement Ratio, represents the ratio of radiation dose needed to cause a certain biological effect under hypoxic conditions compared to normoxic conditions

[7, 8]. In addition, hypoxia is known to contribute to genomic instability and alterations in the DDR signaling [9]. Since the work of Crabtree, Gray and many others (see [10–13] for review), numerous studies have highlighted the increased sensitivity of cancer cells to RT in the presence of oxygen, known as the oxygen enhancement ratio (OER). This has prompted a logical inquiry into its management within clinical settings.

## Reversing Tumor Hypoxia

At a first glance, if hypoxia is associated with poor outcomes in HNSCC patients, the most intuitive approach is to re-oxygenate those tumors. Increasing the percentage of oxygen that is inhaled by cancer patients therefore appears as the most straightforward strategy. The use of hyperbaric oxygen (HBO) is in theory a very relevant approach since it exposes the body to oxygen at higher pressures than normal atmospheric conditions, enabling more oxygen to dissolve in the blood and thus enhancing oxygen delivery to tumors. A systematic review revealed a statistically significant improvement in local control and a reduction in the risk of dying at both 1 year and 5 years after therapy (risk ratio (RR) 0.83, 95% confidence interval (CI) 0.70–0.98) [14]. However, toxicity including barotrauma, ocular manifestations, pulmonary fibrosis and seizures, and logistical issues made the incorporation of HBO therapy into routine practice highly challenging.

Patient discomfort over prolonged HBO schedules is reduced when utilizing normobaric carbogen easily delivered via a mask connected to a tank of carbogen (comprising 95–98% oxygen and 2–5% carbon dioxide). This approach was often associated with vitamin B3 analogue nicotinamide with the goal of reaching the so-called acute hypoxia or perfusion-limited hypoxia (distinguishable from the chronic hypoxia that refers to insufficient $O_2$-diffusion from the chaotic tumor vasculature)

[6]. ARCON (accelerated radiotherapy with carbogen and nicotinamide) was developed as such strategy that combines radiation treatment modifications [15], with the aim of counteracting tumor cell repopulation (through the delivery of radiation doses over a shorter overall period) and both chronic and acute hypoxia (because of carbogen respiration and nicotinamide-induced enhanced tumor perfusion, respectively). While an improved locoregional control was consistently reported with this approach, it is fair to say that the implementation of this approach has not been widely adopted in the world of anticancer radiotherapy. While the gap between the expected effects and the reality of improvement most probably has not sufficiently convinced the radiation-oncology community, this is undoubtedly also largely due to an inability (even more a few decades ago) to identify patients most likely to benefit from this approach (i.e., those with the most hypoxic tumors). Interestingly, since poly-ADP ribose polymerase (PARP) inhibition is now recognized as the mechanism of action of nicotinamide to overcome intermittent vascular constriction, the emergence of novel generations of PARP-1 inhibitors could reignite interest in this approach [16, 17].

Finally, as an additional note about the possibility to reoxygenate tumors, attempts to bolster oxygen delivery by increasing hemoglobin levels using erythropoietin (EPO) have led to disappointing and even detrimental results when combined with radiotherapy [18, 19]. Hypoxia-induced upregulation of the EPO receptor in cancer cells and EPO acting as a growth factor are thought to account for these counterproductive effects [20].

## Killing Hypoxic Cancer Cells

The second possibility to counteract the hypoxia-driven tumor aggressiveness is to directly kill hypoxic cancer cells. Opening an oncology textbook nowadays would instantaneously lead one to consider the hypoxia-inducible factor-1 (HIF-1) as a major target to reach this goal. Indeed, this transcription factor regulates the expression of genes involved in the oxygen-independent glycolytic metabolism that represents a major survival pathway to generate adenosine triphosphate (ATP) when mitochondria are non-functional (because $O_2$ cannot act as the final electron acceptor in the respiratory chain) [21, 22]. Several HIF inhibitors are currently under investigation [23–25]. There are however caveats associated with the use of such compounds since HIF-1 alpha also drives the angiogenesis gene program so that HIF inhibitors could *in fine* exacerbate hypoxia and tumor aggressiveness. Preclinical studies examining strategies that block vascular endothelial growth factor (VEGF), whose the expression is dependent on HIF-1alpha, actually support this statement [26–28]. Nevertheless, it must be mentioned that the tumor vessel normalization paradigm has suggested that a better reoxygenation could result from the pruning of immature angiogenic vessels by VEGF-targeting drugs or antibodies [29]. However, this concept has not translated so far into the launching of new large-scale clinical trials, in part because of the difficulty to assess the occurrence and duration of the available window of increased $pO_2$ [30].

As a matter of fact, the most advanced drugs to kill hypoxic cancer cells are quite old, most often patent-free compounds generally classified as hypoxia-activated prodrugs (HAPs) [31, 32]. These are inactive compounds that are converted into active cytotoxic agents in hypoxic regions of tumors. Nitroimidazoles, including drugs like metronidazole, nimorazole, and misonidazole are the most well-established HAPs, also named "hypoxia modifiers" [33]. Nitroimidazoles are relatively inactive in oxygen-rich environments but undergo enzymatic reduction specifically in regions of low oxygen tension within tumors. The reduced forms of nitroimidazoles are highly reactive and capable of damaging various cellular macromolecules. In particular, the formation of DNA adducts leads to DNA damage and impairment of DNA repair mechanisms within cancer cells [34]. This makes, in theory, nitroimidazoles the perfect radiosensitizers through the exacerbation of the radiotherapy-induced DNA damage and inhibition of repair processes.

In 1998, the DAHANCA 5 phase 3 trial, involving 422 individuals with HNSCC, demonstrated that incorporating nimorazole (a last generation 5-nitro-imidazole) into standard radiation therapy (62–68 Gy, 2 Gy per session, administered 5 times a week) led to enhanced locoregional control at the 5-year mark (49% versus 33%; $P < 0.002$) [35]. Since then, nimorazole usage has been predominantly confined to Danish medical practice. More recently, a randomized trial evaluating nimorazole vs. placebo added to accelerated chemoradiotherapy in HNSCC (DAHANCA 29-EORTC 1219) closed early with 194 of 640 patients recruited, and no differences in 2-year locoregional control probability [36]. In 2023, however, the already lukewarm enthusiasm for this approach was dampened by the publication of a long waited confirmatory trial NIMRAD (Randomised Placebo-controlled Trial of Synchronous NIMorazole Versus RADiotherapy Alone in Patients With Locally Advanced Head and Neck Squamous Cell Carcinoma Not Suitable for Synchronous Chemotherapy or Cetuximab). The NIMRAD phase 3 trial was conducted in 19 UK HNC treatment centers and reported that supplementing intensity-modulated radiation therapy (IMRT) with nimorazole did not improve locoregional control for old and less fit patients with locally advanced HNSCC [37].

This apparently opposite conclusion between the Danish and English trials is the subject of active debates. It's fair to say that it is not the first time that science has faced seemingly opposing results. Instead of pitting the protagonists against each other or focusing only on the latest conclusions while disregarding several decades of work, it seems adequate to examine the determinants of the observed differences. And even if there's difficulty in reconciling the views, leveraging the accumulated data and patient stratification from these studies can help uncover new paths for research.

## DAHANCA 5 vs. NIMRAD

It is worth emphasizing that there are similarities and not only differences between the DAHANCA-5 and NIMRAD clinical studies [35, 37]. The first one is that OS rates were not increased in the nimorazole arm neither in the UK study (NIMRAD)

nor in the Danish study despite locoregional control in the latter (DAHANCA 5: OS 26% vs. 16% 10-year actuarial values in nimorazole vs. placebo group, P = 0.32; OR = 1.32). Secondly, even though there was no gain in OS in the UK study, a trend in favor of nimorazole for primary endpoint of freedom from locoregional progression (FFLRP) could be identified when looking at the Kaplan-Meier curves (NIMRAD: adjusted HR, 0.72 for FFLRP <0.96 for OS).

Next, it is essential to acknowledge differences in the characteristics of HNSCC among the patients included in the studies. Indeed, a more in-depth analysis of the patient cohorts indicates an increased incidence of human papillomavirus (HPV)-positive HNSCC (primarily in the oropharyngeal region) in the UK NIMRAD study (42% *vs.* 25% in the DAHANCA-5 study). Interestingly, in a *post hoc* analysis of DAHANCA-5, for the 25% of patients with HPV-positive tumors, the investigators identified similar locoregional control rates for those who took nimorazole or placebo [38]. Rationale for this difference will be discussed below but indirectly it says that if one restricts the analysis to HPV-negative patients in the NIMRAD study, the number of patients to be considered drops from 338 to 185, among whom half were classified in the hypoxia-high group (i.e., 97 patients out of whom 46 received nimorazole). Also, the use of a predictive 15-gene hypoxia classifier retrospectively applied to the DAHANCA-5 study revealed that only one fourth of the total amount of patients exhibited both a HPV-negative and hypoxia-high status (i.e., 82 out of 323 patients, out of whom 49 received nimorazole) [39]. To this limited number of patients in certain categories, one should add the moderate adherence level to drugs such as nimorazole, and the issue of intra-tumoral heterogeneity leading to potential misidentification of hypoxic *vs.* non-hypoxic tumors (despite recent developments [40]). The message here is not to undermine statistical analyses, but rather to consider through the above figures that one must keep a level head in interpretation.

## HPV-Positive Versus HPV-Negative HNSCC

Although in the last century the principal risk factors for HNSCC were tobacco and alcohol use, the HPV status is nowadays becoming a major contributor of oropharyngeal carcinoma (~40%) [4, 41]. Moreover, the HPV status has been identified as a critical prognostic factor in determining patient response to RT [42]. Indeed, HPV-positive tumors display increased radiosensitivity in comparison with HPV-negative HNSCC, and this is reflected by superior survival rates in HPV-positive patients [43]. To understand, the rationale behind the higher radiosensitivity of HPV-positive tumors, it is worth first to remind the oncogenic potential of the virus. Two viral oncoproteins E6 and E7 were shown to lead to the proteasomal degradation of two tumor suppressor genes TP53 and RB1 [44]. Suppression of p53 is proposed to prevent cancer cells harboring DNA damages to enter apoptosis while Rb suppression mimics the cyclin-dependent kinase (CDK) role and promotes the activation of the transcription factor E2F, thereby leading to uncontrolled cell cycle progression.

Today, this textbook view of the HPV-mediated invalidation of p53 and Rb has to be nuanced. We know indeed that E6 degradation of the p53 protein is not associated with the expected progression into the cell cycle (i.e. failure of cell cycle arrest) as evidenced by reports from several researchers indicating G2/M arrest in response to irradiation [45, 46]. As a consequence, increase in apoptosis was reported, including through a re-expression of p53, according to some authors [45], and elevated levels of residual DSBs according to others [46]. As far as Rb is concerned, the regulation is also more complex because of the associated P16 upregulation resulting from the feedback loop in response to E2F activation [47]. Although P16 expression is often used as a surrogate of HPV infection, the biology of P16 is more complex. Besides the Rb-related pathway regulating the cyclin cascade, there are actually various other non-canonical P16-controled pathways including nucleotide metabolism, protein translation and mitochondrial biogenesis [47], that are thus stimulated in response to HPV infection. Recently, the group of Skinner showed that P16 was associated with an increased activity of the transcription factor SP1 (specificity protein 1), leading to increased transcription of HUWE1 which is a ubiquitin ligase, and consecutive degradation of the deubiquitinase USP7 and one of its target, TRIP12 [48]. TRIP12 prevents excessive spreading of ubiquitinated chromatin at damaged chromosomes so that its downregulation in HPV-positive HNSCC cells induces a reduction in the repair capacity of DNA double-strand breaks and thus supports the improved response to radiotherapy [49].

Altogether, these data support a model wherein HPV-positive HNSCC patients do not benefit from nimorazole treatment, not because of inherent differences in hypoxia response but instead through an overall higher radiosensitivity of HPV-positive cancer cells (i.e., a reduced capacity to repair DNA damages). In other words, even though like for other tumors, the OER does apply to HPV-positive HNSCC, it is not synonymous of an increased activity of hypoxia modifiers (Fig. 13.2). Also, because of the increased response to irradiation and thus enhanced cancer cell killing (for the same radiation dose), HPV-positive tumors undergo significant reoxygenation, rendering hypoxia modifiers ineffective in their sensitization role (Fig. 13.3).

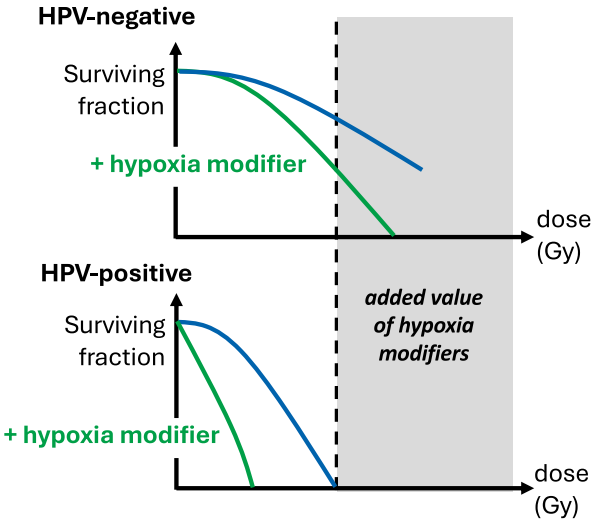


**Fig. 13.2** Higher radiosensitivity of HPV-positive HNSCC but not the absence of OER accounts for the apparent lack of activity of hypoxia modifiers. Graphs depict the dose-survival curve in the absence (blue) or the presence (green) of hypoxia modifier for HPV-negative (top) and HPV-positive (bottom) HNSCC

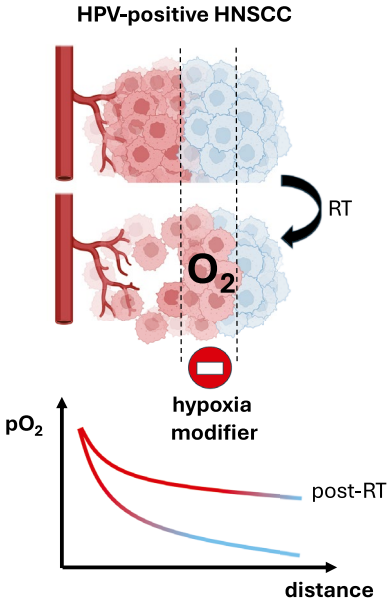


**Fig. 13.3** HPV-positive cancer cell killing by radiotherapy (RT) induces a pronounced tumor reoxygenation, rendering hypoxia modifiers not or poorly effective. Bottom graph depicts the less steep relationship between $pO_2$ and distance from blood vessels after radiotherapy

## Oxygen Consumption Inhibitors as a Reoxygenation Strategy

The above conclusion that says that after each radiation fraction, surviving HPV-positive cancer cells become more oxygenated is actually nothing more than a justification for the fractionation of current RT. The emphasis on reoxygenation's role in radiosensitization has diverted attention from the biological inquiry into how

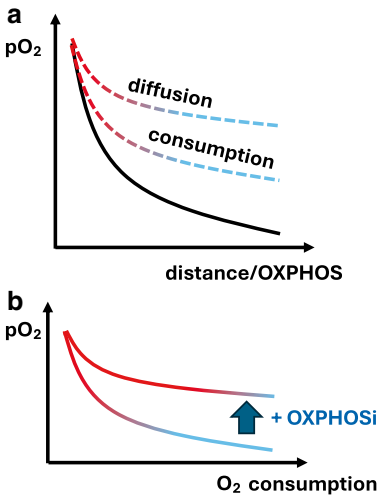


**Fig. 13.4** Cancer cell respiration as the primary contributor to tumor hypoxia. (**a**) Limitations in $O_2$ diffusion but also in $O_2$ consumption contribute to hypoxia. (**b**) OXPHOS inhibitor promotes reoxygenation and enhance tumor radiosensitivity

cancer cells utilize the increased availability of oxygen. The most obvious response is that they use oxygen to fuel their mitochondrial metabolism (Fig. 13.4). This seemingly obvious claim accounts for a paradigm shift that says that instead of harnessing hypoxia modifiers to take advantage of a low $pO_2$, in a tumor, an appealing alternative could involve inhibiting oxygen consumption and working synergistically with the reoxygenation triggered by a fractionated radiotherapy regimen. Interestingly, this idea is far from being new since reduction in the oxygen consumption rate was already predicted several decades ago to be 30-fold more efficient in reducing hypoxia than increasing oxygen delivery by methods such as breathing hyperbaric oxygen or increasing vascular perfusion [50–52].

Oxygen consumption inhibitors were already proposed as potential radiosensitizers by Durand and Biaglow at the end of the 1970s [53, 54]. Their observations were originally made with mitochondrial poisons used on cancer cells cultured *in vitro*. More recently, we discovered that inhibiting the mitochondrial pyruvate carrier which prevents pyruvate from fueling the Krebs cycle with acetyl-coenzyme A and subsequently supporting oxidative phosphorylation, (OXPHOS) significantly enhances the radiosensitivity of tumors *in vivo* [55]. This example is not isolated, and there is actually much evidence documenting that radiosensitizing effects can be obtained upon inhibition of one or several complexes of the mitochondrial electron transport chain or more generally via a reduction in the oxygen consumption rate. Those strategies include metformin [56, 57] but also phenformin [58], metformin conjugated with mitochondria-targeting triphenylphosphonium cation [59] and a series of compounds with a large variety of structures including papaverine [60], Bay 87-2243 [61, 62], IACS-010759 [63], carboxyamidotriazole derivatives [64], atovaquone [65], evofosfamide/TH-302 [66–68] and even arsenic trioxide [69].

Another set of intriguing data stems from the recent introduction of anti-programmed cell death-1 (PD-1) therapy for HNSCC patients, which, despite some successes, shows limitations for a significant portion of patients [70, 71]. Analysis

of recurrent or metastatic HNSCC has revealed that hypoxia was associated with poor clinical efficacy of anti-PD-1 antibodies [72]. Strikingly, hypoxia was not identified as the cause of treatment resistance but rather resulted from increased oxidative metabolism in anti-PD1-resistant HNSCC tumors [72]. This observation indirectly confirms that the primary driver of tumor hypoxia is cancer cell respiration (i.e., oxygen consumption) rather than inadequate oxygen supply.

## Conclusions

Clinical studies utilizing HAPs and post-hoc analyses have shed light on intrinsic differences in radiosensitivity. In particular, the ability to repair DNA damage appears to be central in justifying the difference between HPV-positive oropharyngeal carcinomas and HPV-negative HNSCC. This finding now enables the identification of pathways in HPV-positive tumors (contributing to enhanced radioresponse) that could be targeted pharmacologically in HPV-negative cancers to improve patient outcomes. As such, a notable example derived from the mechanistic dissection of radiosensitivity in HPV-positive cancers is the use of USP7 inhibitors (currently in development) to inhibit the activity of TRIP12 in HPV-negative cancers [48]. It can also be anticipated that the development of various classes of drugs capable of interfering with DDR will take advantage of the increasingly detailed understanding of radioresistance mechanisms in HPV-negative HNSCC. Particularly, the advancement of biomarkers should enable administration of these drugs to the most suitable subpopulations of HNSCC patients (i.e., where the risk balance between antitumor efficacy and deleterious effects in irradiated healthy tissues is favorable). Lastly, the increasing interest in tumor metabolism has led to the development of drugs that may represent valuable tools when combined with RT. While these medications may not directly eliminate cancer cells that exhibit high metabolic plasticity, they could indeed induce synthetic lethality by forcing them to depend on OXPHOS. Recent findings on the mitochondrial activity in tumors, long underestimated due to misinterpretation of the Warburg effect [21], suggest that strategies promoting tumor reoxygenation by inhibiting mitochondrial Krebs cycle or respiratory chain are more promising than approaches aiming to harness tumor hypoxia with HAPs. Active research in the field of HNSCCs will certainly keep injecting a breath of fresh air into the study of tumor hypoxia and its clinical implications.

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# Chapter 14
# Hyperthermia: A Game Changer in the Management of Head and Neck Cancer?


**Anthony Kong**

## Background

Locoregionally advanced head and neck squamous cell carcinoma (LA-HNSCC) is treated with a combination of surgery, radiotherapy and/or chemotherapy depending on the tumour subsites [1]. Concurrent chemotherapy with radical radiotherapy has been shown to increase survival in LA-HNSCC but the survival benefit decreases with increasing age with no significant survival benefit in those patients over the age of 70 years, as suggested by the individual patient-based meta-analysis of Pignon et al. [2]. In addition to concurrent chemotherapy, the combination of cetuximab with primary radiotherapy has also been shown to increase survival compared to radiotherapy alone [3]. However, no survival outcome benefit was demonstrated when cetuximab was added to chemoradiation compared to chemoradiation alone [4].

The question was previously raised whether there would be a difference between concurrent chemotherapy compared to concurrent cetuximab with radiotherapy (RT) in LA-HNSCC. A randomized phase II study of cetuximab and radiotherapy versus cisplatin and radiotherapy for LA-HNSCC was conducted but the study was terminated early due to poor accrual [5]. Surprisingly, it was found that concurrent cetuximab with radiotherapy lowered compliance rate and increased acute side effects, especially skin and mucosal toxicities [5]. In addition, the 2-year local control rate and overall survival were lower in the cetuximab arm, 53% and 68% versus 80% and 78%, respectively, in the cisplatin arm. However, these results were not statistically significant due to early trial termination and no definite conclusion can be made [5]. In view of the better prognosis of patients with human papillomavirus (HPV) positive oropharyngeal carcinomas, there has been an initiative in decreasing

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the treatment intensity, including replacing concurrent chemotherapy in these patients. However, two large randomised trials, De-ESCALaTE and RTOG 1016, showed an inferior overall survival in those patients treated with concurrent cetuximab with radiotherapy compared to concurrent cisplatin with radiotherapy in HPV positive oropharyngeal cancer patients [6, 7]. Thus, concurrent platinum-based chemotherapy with radiotherapy remains the current standard treatment for both HPV-positive and HPV-negative LA-HNSCC. Cetuximab in combination with radiotherapy is also an approved treatment, usually reserved for patients with poor renal function and those not suitable for concurrent platinum-based chemotherapy.

Around 40% of HNSCC patients that are currently treated by radiotherapy are over 70 years. Although these patients were not excluded from the initial radiotherapy and cetuximab combination trial [3], many oncologists would not offer concurrent cetuximab with radiotherapy for older patients in view of a concern of increased skin toxicities [8, 9] and the subsequent subgroup analyses showed that the benefit of cetuximab is confined to patients who are less than 70 years old [10]. Therefore, most of the older patients are offered primary radiotherapy alone for LA-HNSCC in view of no survival benefit from primary concurrent chemoradiotherapy over radiotherapy alone in these patients. There have been initiatives to see whether hypoxia radiosensitizer could be used to enhance the radiotherapy effect and survival in these patients. One of these agents is nimorazole, which is used to treat anaerobic bacteria and protozoan infections. Previously, DAHANCA 5–85 study showed that the addition of nimorazole to radical radiotherapy resulted in a significantly better loco-regional control rate compared to the placebo group in patients with supraglottic larynx and pharynx carcinoma [11]. However, the recent results from the NIMRAD study, a randomised placebo-controlled phase 3 trial showed that the addition of nimorazole to radiotherapy did not improve locoregional control or overall survival compared to radiotherapy alone in patients with LA-HNSCC not suitable for concurrent chemotherapy or cetuximab [12]. Therefore, there remains an unmet need to develop other strategy that can help to improve the outcome for these locally advanced HNSCC patients who would otherwise receive radiotherapy alone due to their age or if platinum-based chemotherapy is deemed to be inappropriate.

## Hyperthermia as a Radiotherapy, Chemotherapy and Immunotherapy Sensitizer

Hyperthermia (thermotherapy) treatment applies heat to the tumour and surrounding tissues to increase the temperature to about 40–44 °C [13]. In preclinical settings, hyperthermia has been shown to have direct thermal cytotoxicity effect by inducing DNA double-stranded breaks, although most of the anti-tumour effects are thought to be due to augmentation of DNA damaging treatments [14, 15]. Several publications have described the rationale of hyperthermia to be a potent radiotherapy and chemotherapy sensitizer through various mechanisms including an increase

of vascular blood flow, reduction of hypoxia and inhibition of the DNA repair enzymes [16, 17]. One of the key aspects is that hyperthermia limits the ability of the tumour cells to repair DNA damage induced by radiotherapy and/or chemotherapy through degradation of BRCA [18]. It can also modulate tumour microenvironment and potentially improve response to immunotherapy [19].

The beneficial effect of adding hyperthermia to radiotherapy in various cancers has been known for some time. Datta et al. (2015) summarized the outcome of various randomised and non-randomised clinical studies of the combination of hyperthermia with radiotherapy for different tumours, including breast, cervix, head and neck, rectum, urinary bladder, oesophagus, cutaneous melanoma and others [20]. In 38 clinical trials with 1717 patients receiving radiotherapy alone compared with 1761 patients treated with radiotherapy and hyperthermia, there was a higher overall complete response (CR) rate of 54.9% with hyperthermia plus radiotherapy compared with a rate of 39.8% with radiotherapy alone, of which 9 studies were in head and neck cancers (a CR of 75.3% in 364 patients treated with hyperthermia and radiotherapy versus a CR 50.3% in 353 patients treated with radiotherapy alone) [20]. No significant increase in acute or late toxicity with the addition of hyperthermia to radiotherapy was evident from these studies [20]. A further systematic review and meta-analysis further confirmed the superior efficacy with the combination treatment, an overall CR of 62.5% with hyperthermia plus radiotherapy compared with 39.6% with radiotherapy alone [21]. However, most oncologists do not regard these studies or the systemic review as strong enough evidence to support the routine use of hyperthermia in head and neck cancers. This is because most of these published randomised controlled trials only included a small number of patients and were conducted many years ago using older radiotherapy techniques and hyperthermia technologies with inefficient focused heating and poor temperature monitoring, which could result in a poorer outcome compared to the current standard of care.

## Technologies to Deliver Hyperthermia for Head and Neck Cancers

To achieve regional hyperthermia in the range of 40–44 °C heating, several technologies are available, including high intensity focused ultrasound (HIFU), scanned focused ultrasound (SFUS), radiofrequency (RF), capacitive systems and infrared (IR) [22–24]. However, none of them is good in heating the deep-seated tumours efficiently and precisely in the head and neck region.

The IR technology including water-filtered Infra-Red A (wIRA) machine has been used widely for superficial recurrent cancers such as locally recurrent breast cancers with great efficacy when combined with (re-)irradiation [25] but it is of limited use in head and neck cancers due to its penetration depth to only around 2 cm. The capacitive heating technologies have limited steering capabilities as they only work with two electrodes, which creates a risk on nearby sensitive organs [23, 24]. The HIFU or SFUS is a powerful technique, but it is limited in size of the

tumour (stage I and early stage II) [23, 24]. The current existing RF based heating machines on the market use a lower frequency and are unable to focus the heat on the tumour alone while avoiding heating nearby sensitive organs. Thus, these current available technologies will not provide the ability to treat deeper seated tumours in the head and neck area.

## Deep Hyperthermia for Head and Neck Cancers

One of the promising RF based technologies that could potentially provide a commercial solution to heat the head and neck tumour more efficiently is the deep hyperthermia system from Sensius. The initial concept of thermotherapy was developed at the Erasmus Medical Centre in Rotterdam, the Netherlands. With this first prototype called HyperCollar, 46 patients were treated in a feasibility study using the HyperCollar deep hyperthermia system in combination with radiotherapy for patients with recurrent and locally advanced patients [26]. A second, improved prototype, the HyperCollar3D was used to treat 22 head and neck cancer patients and the results showed a 12-week complete response rate of 81.8% with a cumulative grade 3 toxicity of 39.2% in patients with recurrent HNSCC treated with a combination of thermotherapy and radiotherapy [27]. However, these are retrospective studies including mainly recurrent HNSCC patients who had thermotherapy with re-irradiation as a compassionate use setting and were not done in a prospective clinical trial setting [26, 27]. The Erasmus Medical Centre Rotterdam will be starting a phase 1b dose-finding study called TANCA-I in 2025, combining radical radiotherapy with thermotherapy using HyperCollar3D for HNSCC patients not suitable for concurrent chemoradiation. The primary objective is to find the maximum tolerated dose (MTD), which is based on the energy delivered to the target and the patients' weight.

Sensius has further developed the HyperCollar 4D as a commercial solution which offers more comfort and targeted heating solution (see Fig. 14.1). They have stated that this would be the only technology currently available in controlling the energy sufficiently to create a conformal heating pattern in the head and neck area [28]. The Hypercollar 4D (also called FocusCollar) system produces radiofrequency waves (434 MHz) that are directed to the tumour by the head and neck applicator. The waves are directed by 20 antennas that are in the applicator, so the tumour is heated conformally in 3D, based on a CT scan that is also used for the radiotherapy. The treatment planning software steers the antennas, and the energy is aimed only at the tumour, whilst not heating the organs at risk and tissue nearby. The treatment requires a steady state tumour heating of 60 minutes at 42 °C. After treatment, the results are entered into the patient's dossier.

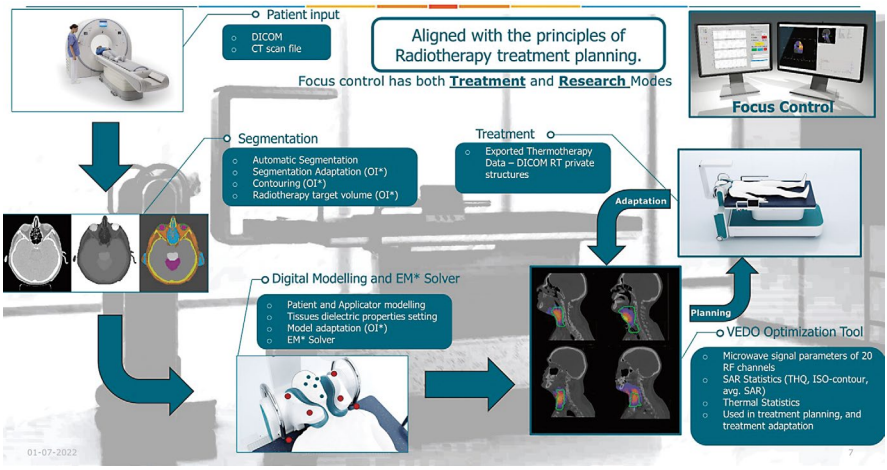


**Fig 14.1** The integration and the workflow of the FocusSystem in applying deep hyperthermia (thermotherapy) in combination with radiotherapy will be aligned with the standard radiotherapy pathway for head and neck cancer patients. The thermotherapy treatment can be performed by the radiotherapy treatment radiographers after minimal extra training. *OI** Operation Interaction, *EM** Electromagnetic. (Figure is kindly provided by Sensius)

## Potential Clinical Trials in Thermotherapy

In order to change clinical practice and to introduce thermotherapy as standard of care in head and neck cancers, large well-conducted randomised trials are required to demonstrate an increased efficacy and locoregional control as well as survival benefit with the addition of thermotherapy to radiotherapy compared to radiotherapy alone in LA-HNSCC. A potential first trial could be a randomised phase 2/3 trial to assess the improvement in locoregional control rates and survival when thermotherapy is added to radical radiotherapy compared to radical radiotherapy alone in HNSCC patients not suitable for concurrent chemotherapy. The other potential trial is a randomised phase 2/3 trial comparing the overall response rate and survival outcomes between combination of thermotherapy plus palliative radiotherapy with palliative radiotherapy alone in patients with incurable HNSCC. These two trials could be combined into one as separate cohorts under a platform trial, and this would be able to demonstrate whether thermotherapy could improve the efficacy and survival outcomes when added to primary radical radiotherapy and palliative radiotherapy.

There are many other potential clinical trials that could be conducted to demonstrate how thermotherapy may make a difference in head and neck cancers. In the view of the good prognosis of HPV positive HNSCC, does thermotherapy play a role in de-escalation of the treatment and replacing concurrent chemotherapy with radiotherapy with similar efficacy and less side effects? In this case, it will need to be a head-to-head comparison between concurrent chemoradiation versus

thermoradiotherapy in a randomised phase 2/3 trial. However, there will invariably be concern that thermoradiotherapy would be inferior to concurrent chemotherapy, given the negative results of De-ESCALaTE and the RTOG 1016 trials previously [6, 7]. This de-escalation study may need to wait until it can be demonstrated that the addition of thermotherapy results in a superior outcome in those chemotherapy ineligible patients who would otherwise receive radiotherapy alone. And for HPV negative HNSCC with poor prognostic features, could the addition of thermotherapy to concurrent chemoradiation improve locoregional control and overall survival? The proposed randomised phase 2/3 study would involve the addition of thermotherapy to chemoradiation versus chemoradiation alone in HPV negative HNSCC patients, which could include patients undergoing both primary and adjuvant chemoradiation. Lastly, since thermotherapy could also modulate tumour microenvironment [19], could thermotherapy potentiate first-line pembrolizumab treatment in recurrent and metastatic HNSCC patients? This could be a phase 2/3 trial to assess whether the combination of thermotherapy plus pembrolizumab could improve survival outcomes compared to pembrolizumab alone in patients with programmed death ligand-1 (PD-L1) positive tumours (combined positive score (CPS) $\geq$1). This author and several other head and neck oncologists internationally have had discussions to consider the design of these different potential randomised controlled trials above. There are many challenges ahead including establishing adequate FocusCollar centres and acquiring adequate funding to do joint international studies involving thermotherapy. However, a joint international effort is already underway to make this possible and this represents an exciting time for thermotherapy in head and neck cancers.

## Conclusions

Hyperthermia is a potential game changer in the management of head and neck cancers given its ability to sensitize radiotherapy, chemotherapy and immunotherapy with minimal increase of additional severe toxicities. However, new evidence from well-conducted large randomised controlled trials is required to demonstrate the survival benefit and safety from the addition of thermotherapy to the standard of care before this would result in a practice change in head and neck cancers. A concerted international effort is required and is already underway to conduct such randomised controlled trials.

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# Chapter 15
# The Evolving Landscape of Prognostic Factors in HPV-Related Oropharynx Cancer

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## Introduction

Risk-tailored approaches are the backbone of contemporary clinical research and treatment. With the recognition of a significantly better prognosis in most patients with HPV-mediated oropharyngeal carcinoma (HPV+ OPC) compared to traditional smoking-related/HPV-negative disease, great effort has been devoted to develop management algorithms specifically tailored to HPV+ OPC patients. However, while a variety of new treatment options has emerged, little progress has been made in optimizing management of HPV+ OPC from the stand-point of improving prognosis for any of its subgroups or reducing long-term toxicity across the disease. Despite a few successful phase II trials, none of the phase III trials have

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been successful so far [1–3]. This highlights the importance of careful identification of prognostic factors and derivation of risk stratification models that enhance targeting of different subgroups in clinical trials, as well as appropriate integration of emerging therapeutics with traditional approaches.

Constructing appropriate risk stratification models requires identifying and understanding prognostic factors, accurate analysis of appropriately assembled datasets, and interpreting their clinical applicability in the relevant setting. In the context of HPV+ OPC, the impact and appropriateness of many known prognostic factors derived empirically from smoking-related head and neck cancer (HNC) require reappraisal. It is also important to recognize promising emerging prognostic anatomic and biologic factors that may be crucial for risk stratification and treatment optimization in the near future.

This chapter summarizes recent updates on prognostic factors and risk stratification from the standpoint of pre-treatment, baseline prognostic factors, and dynamic factors available after commencement of treatment.

## Pretreatment Factors

### *Reappraisal of Smoking in Risk Stratification*

Risk-stratification model development is essential to accurately depict subgroups with distinct outcomes, and must especially address what prognostic factors to be included in the model. The first report of the RTOG 0129 trial [4] confirmed differences in overall survival (OS) in two types of oropharyngeal carcinomas: HPV+ and HPV-negative (HPV–). This was an evolution of the initial observation by Fakhry et al. [5] who performed genomic DNA analysis of oncogenic HPV types 16, 33, or 35 in patients included in an Eastern Cooperative Oncology Group (ECOG) phase II trial and identified that, in patients with OPC, tumour HPV status is strongly associated with therapeutic response and survival. RTOG 0129 also showed that heterogeneity in OS existed in OPC although much of the adverse behaviour is due to the inclusion of HPV-negative patients in the study which is no longer relevant in specific discussions of HPV+ OPC today, notwithstanding additional variability in its behaviour. Recursive-partitioning analysis (RPA) that included any smoking pack-year (PY) exposure as a parameter in the model, classified all HPV+ OPC (including T4 and N3 cases) with less than 10 PY smoking together with those >10 PY with TNM7 N0-N2a diseases as low-risk of death subset. An additional caveat of this report is that the outcome analyses were restricted to reporting OS and progression-free survival (PFS), which both contain death from any cause as events, and do not differentiate index-cancer specific deaths (requiring intensified or improved treatment) from competing-risk mortality (requiring other management strategies, such as better management of comorbidities and smoking cessation). Specific behaviour of the disease itself, such as locoregional control (LRC) and distant metastasis (DM), as well as the timing of smoking exposure (current vs

former vs during chemo-radiotherapy) are also not addressed in this report. Finally, the influence of an important host factor, i.e. age of the patient, remained underemphasized despite having as strong an effect on survival as smoking.

The hazardous influence of smoking on OS in any medical context is ubiquitous and certainly also applies in HPV+ OPC patients where its influence remains enigmatic. Its "impact" is significantly confounded by competing risks that are often non-oncologic and unrelated to the index primary. The potential contributions to inferior OS in HPV+ smokers include the following:

1. *Competing mortality risk from smoking*, such as increased risk of late toxicity, second primary, chronic cardiac and pulmonary disease, social problems, etc., remain a continued challenge in many medical spheres [6–8]. To overcome these detriments, better management of comorbidities, and continued prior and later avoidance to mitigate ongoing risk caused by smoking should be more important.
2. *Treatment intolerance due to comorbidities*. Although the frequency of severe mucositis was similar, smokers during radiotherapy experienced prolonged hospitalization to support healing compared to non-smokers [9].
3. *Reduced radiotherapy efficacy (hypoxia) in continuing smokers during (chemo-) radiotherapy* [10–12]. It is well-known that active smokers have higher circulating carboxyhemoglobin which is inversely related to tumour oxygen level [13]. The smoking-induced tumour hypoxia due to increased carboxyhemoglobin appears to significantly reduce radiosensitivity as originally reported by Browman et al. in a landmark paper in 1993 from a randomised trial addressing two strategies of loco-regional management of locally advanced head and neck cancer, prior to the known influence of HPV [11]. Quitting smoking has been documented to quickly reverse this process [10]. The detrimental effect of active smoking during radiation has now extended to HPV+ OPC. In a *post hoc* pooled analysis of the RTOG 9003 and 0129 trials, Gillison et al. [14] reported that active smokers during radiotherapy had the highest mortality risk which mirrors the original observation by Browman and colleagues. Similarly, a single institution study of 484 HPV+ OPC patients found that active smoking status at diagnosis is a stronger predictor than smoking pack-years for OS and PFS [15]. This supports the importance of smoking cessation prior to and during radiotherapy. An additional study of HNC (also not HPV-specific) affirmed that those who quit smoking during chemoradiation (CRT) had significantly higher disease-free survival (DFS, a.k.a. PFS), lower acute toxicity, and a lower rate of permanent tracheostomy [16]. A meta-analysis [12] also demonstrated that those who ceased smoking had better locoregional control (LRC) and OS vs those actively smoking during radiation. Parenthetically, for those who are unable to quit smoking, an alternative and realistic option would be to choose primary surgery when feasible and appropriate to balance oncologic and functional outcomes, since surgical series have not demonstrated any impact of smoking effect on disease specific outcomes [17, 18].
4. *'Hybrid' tumour (driven by both HPV and smoking)/aggressive phenotype*. Weinberger et al. [19] proposed a three-class classification for OPC based on

p53 (wild type vs mutation), p16 (positive vs negative), and HPV DNA (positive vs negative): *Class I*—p53 mutation, and both p16 and HPV DNA negative, presumably driven by tobacco exposure, *Class II*—p16-negative but HPV DNA-positive, and p53 mutation, hypothetically a 'hybrid' tumour driven by both tobacco and HPV, and *Class III*—both p16 and HPV DNA positive and p53 wild-type, presumably driven by HPV. While *Class III* phenotype had the best outcomes, *Class II* phenotype had similarly poor outcomes compared to tobacco-driven *Class I* OPC. However, HPV DNA positivity does not necessarily prove the existence of an HPV-driven tumour, since an oncogenic signal (e.g. presence of HPV mRNA) was not evaluated. It is possible that the presence of HPV DNA could represent a by-stander HPV infection in *Class II* tumours. Furthermore, while exploring the potential that smoking might alter the character of HPV-related OPC during its pathogenesis, Mirghani et al. [20] performed targeted next-generation sequencing in 62 HPV+ OPC tumours (37 non-smokers and 20 smokers), and found no difference in mutational rate or mutational pattern according to smoking pack-years (>20 vs ≤20) and smoking status (smokers vs non-smokers).

5. *Altered tumour / host microenvironment (e.g. immune suppression)*. Although there is no evidence that smoking alters the biology of HPV+ OPC, recent data suggests that tobacco use may alter the tumour-microenvironment (TME), and appears associated with an "acquired" immune desert TME phenotype. Strong evidence shows that tobacco smoking impacts both innate and adaptive immunity [21]. Wahle et al. [22] performed integrative genomic analysis on 43 HPV+ OPC, and found that tobacco use at the time of OPC diagnosis was associated with lower tumour infiltrating lymphocytes (TIL) in the primary tumour TME, and also found no evidence of increased mutational burden or recurrent oncogenic mutations. Thus, it is plausible that a major biologic influence of tobacco exposure may relate to TME immunosuppression.

In summary, it is beyond doubt that tobacco smoking is detrimental to HPV+ OPC patients' general health and results in untimely death compared to non-smokers. When analysing outcome, this will impact on end-points such as PFS which includes death from any cause and deliberate attention must be given to the manner in which the tumour, as opposed to the host/patient is affected. Evidence has existed for some time that active smoking creates a hypoxic environment capable of reducing radiosensitivity and compromises normal tissue recovery. Recent findings suggest that it may also create an immunosuppressed TME that could potentially inhibit response to anti-PD-1 therapy. All these could adversely affect OS and PFS/DFS/event-free survival (EFS). Smoking cessation should be recommended to all active smokers at the time of consultation. For those who cannot quit smoking, an alternative strategy, such as using surgery as the primary treatment modality, might be considered in the decision-algorithm to avoid active smoking related reduced radiosensitivity, compromised normal tissue tolerance, and a detrimental effect on the TME.

## *Molecular Profiling and Immune-Based Risk Stratification*

HPV+ OPC is known for a more robust activation of several immune signalling pathways compared to its HPV-negative counterparts. A gene expression analysis of 39 OPC patients (24 HPV+ and 15 HPV–) showed that HPV+ OPC is more likely to be "T-cell-inflamed" manifested as increased TILs, CD8 T cells, and cytotoxic cells, while HPV-negative OPC is more likely to be "T-cell-non-inflamed" [23]. Several reports have shown that the quantification of TILs and PD-L1 expression is associated with favourable outcomes [24–26].

Despite HPV+ OPC being generally an immunogenic tumour, heterogeneity in TME exists in HPV+ OPC. CD103 is a phenotypic marker of tumour resident memory T cells ($T_{RM}$) cells, which promotes T-cell effector functions. Rischin et al. [27] analysed HPV+ OPC patients enrolled in the TROG 12.01 and De-ESCALaTE randomized trials, and found that the subgroup with high CD103 intratumoural immune cell (ITIC) expression had much better prognosis compared to those with low CD103 expression. Solomon et al. [28] reported that CD103 could further identify patients with favourable outcomes within each TNM Stage Group. Some of these subgroups, defined primarily by molecular characterisation could represent viable candidates for de-intensification clinical trials. Solomon et al. [29] also reported that the subgroup of HPV+ OPC with both high intratumoural PD-L1 expression and abundant CD8+ TILs in HPV+ OPC had better prognosis. Zeng et al. [30] developed a UWO3 score based on the gene expression of CD3E, ZAP70 and IRF4 in tissue-infiltrating immune and stromal cells on formalin-fixed paraffin-embedded (FFPE) HPV+ OPC tissue blocks, and classified tumours into 3 TME immune classes: "immune rich" (UWO3 $\leq$2), "mixed" (UWO3 between 2 and 2.5), and "immune desert" (UWO3 >2.5). Patients with "immune rich" (UWO3 $\leq$2) HPV+ OPC had superior DFS compared to "mixed" and "immune desert" patients. The authors suggest therapeutic avenues that could potentially involve substituting chemotherapy with immunotherapy for treatment de-escalation in the "immune rich" group or using neoadjuvant immunotherapy to increase T-cell density within the "immune desert" tumours, in addition to subsequent concurrent chemotherapy after the induction phase. Mehanna et al. studied 985 OPC patients [31] and developed a multivariable prognostic-predictive classifier that combined clinical and molecular biomarkers (including survivin and TIL) to predict outcomes as well as facilitate treatment selection with particular emphasis on those patients who do not enjoy the same success with contemporary treatments. Table 15.1 summarizes selected studies regarding immune-based risk stratification.

In summary, there is heterogeneity in TME and immune profiles (rich vs desert) that provide new opportunities for immune-based risk stratification beyond current anatomic TNM classification and other prognostic models, especially when confounding effects could influence varied end-points (e.g. the effect of smoking was discussed in detail earlier). Furthermore, this may also provide opportunities for better treatment selection. However, assay robustness and clinical utility remain to be validated.

**Table 15.1** Selected studies on immune-based risk stratification in HPV+ OPC

| Author, Year | Study Description | Findings |
|---|---|---|
| Atipas, 2023 [94] | N = 160 (p16+ 27; p16–: 133)<br>Retrospective<br>Population: OPC 2012–2018<br>CD8+ TIL divided into CD8 ≥10%, CD8 ≥5% but <10%, CD8 ≥1% but <5% and CD8 <1%<br>PD-L1 evaluated using into three groups: CPS <1, CPS ≥1 but <20, and CPS ≥20.<br>Tumour environment into 4 main types per PD-L1 and CD8+ TIL status:<br>Type 1 (PDL1+/TIL+); Type 2 (PDL1–/TIL–); Type 3 (PDL1+/TIL–); Type 4 (PDL1–/TIL+) | Median FU: 1.38 years (range, 0.06–8.26)<br>OS was associated with higher density of CD8+ TIL (various cutoffs), but not with PD-L1 expression<br>Within the p16– group: higher density of CD8+ TIL (≥1% cut-off) had higher OS<br>MVA: lower OS with higher stages, p16- status and low CD8+ IL density<br>Type I tumour environment had the best OS, followed by type IV, II and III |
| Hewavisenti, 2020 [95] | N = 62 (35 HPV+; 27 HPV–, TCGA database)<br>Determining the prognostic value of Trm T cells with in (T1–T4 N0–3 M0) OPC by HPV status.<br>Multiplex quantitative IHC staining for CD103 and CD 68 (both associated with CD8+ Trm T cells).<br>Examined the accumulation of CD103+CD8+ T cells within the tumour and the stroma. | Median FU 65.5 months.<br>More CD103+CD8+ T cells in HPV+ vs HPV– OPC<br>In HPV+ OPC, higher OS with higher intratumoural CD103+CD8+ T cells<br>In HPV+ OPC, higher OS with high vs low CD103+CD8+ T cell count, but both remains favourable.<br>HPV– OPC with high CD103+CD8+ T cell count vs HPV+ OPC had similarly good OS<br>Intratumoural Trm level was a significant predictor for OS on MVA |
| Rischin, 2022 [27] | N = 304 (159 TROG 12.01; 145 De-ESCALaTE).<br>Compared CD103+ ITIC abundance (≥30% vs <30%)<br>Compared CD8+ TIL (≥30% vs <30% intratumoural and stromal) | Median follow up 3.2 years<br>RT/CETUX patients: higher 3-year FFS and OS in patients with high vs low CD103+ ITIC count<br>RT/CIS patients: No significant difference in 3-year FFS between high vs low CD103+ ITIC count |

(continued)

**Table 15.1** (continued)

| Author, Year | Study Description | Findings |
|---|---|---|
| Tosi, 2022 [23] | N = 39<br>Population: Node positive OPSCC treated with upfront surgery.<br>RNA extracted from primary tumour for PanCancer Immune Profiling panel used to measure the expression of 770 immune-related genes.<br>Multiplex immunofluorescence performed on primary and lymph node metastases evaluating CD68, CD8, FoxP3, CD 163, CD 103, PD-1, PD-L1, CK, CTLA-4, CD4, CD8 and HLA-1.<br>Multispectral imaging, cell densities, cell percentages and cell to cell distances analysed for each sample. | Improved DFS was seen in primary lesions that are heavily infiltrated with intra-tumoural CD8+PD-1+, CD8+103+PD-1, PD-L1+ cells<br>Higher frequency of PD-L1+ macrophages within a 20 μm radius from PD-1 CTL was associated with improved DFS<br>Higher DFS in HPV+ patients with higher densities of stromal or intratumoural CD8+, intratumoural CD4+ T cells, total T lymphocytes, CD68+ macrophages and CD163+ TAMs in primary tumour and higher % of cytotoxic T lymphocytes within a 20 μm radius to macrophages<br>Lower DFS in patients with HPV– tumour with higher density of CD68+CD163+ cells and higher percentage of CD8+ T lymphocytes within a 20 μm radius from CD163+ macrophages |
| Snietura, 2020 [96] | N = 85<br>Evaluate impact of the intensity of macrophage infilters of the M1/M2 and M2 phenotype on the clinical outcome of patients with OPSCC.<br>Tumour stained for CD68 pan-macrophage marker predominantly in inflammatory M1 phenotype macrophages.<br>Tumour stained for CD168 as a marker for M2 phenotype. | N = 45 PV+ and N = 40 HPV−<br>HPV+ more intense CD68+ and CD163+ cells in the tumour stroma but did not differ intratumourally.<br>In HPV–, higher density of CD68+ infiltrate was seen in patients without (vs with) nodal recurrence<br>Better LRC, MFS, and OS was seen in HPV– patients with infiltrates of low compared to high intensity CD163+ macrophages |
| Solomon, 2019 [28] | N = 366 [test cohort: N = 189); validation cohort: N = 177)]<br>IHC for p16, CD103 and CD8<br>Semi-quantitatively scoring proportion of positively stained cells in both intratumoural and stromal adjacent to the tumour nest of the total number of cells in each compartment as 0%, 1%, 5%, 10%, 20% and 30–100%<br>Best cut off for CD103+ was determined to be (<30% vs ≥30%) | Median FU 5.2y and 4.6y for cohort 1 and 2<br>In the test cohort: higher OS was seen with high vs low (≥30% vs <30%) intratumoural CD103+ cell. This was validated in cohort 2.<br>Abundance of stromal CD103+ cells did not correlate with outcome.<br>Post hoc analysis: High CD8+/high CD103+ showed superior OS vs high CD8+/low CD103+ ($p < 0.001$).<br>Tumour with high CD103+ correlated to higher intratumoural immune cell PD-L1 expression than low CD103+ tumours.<br>Adjusting for smoking, stage and age, CD103+ cell abundance provided additional prognostic value on OS. |

(continued)

**Table 15.1** (continued)

| Author, Year | Study Description | Findings |
|---|---|---|
| Zeng, 2022 [30] | N = 906 (6 cohorts)<br>Created immune score (UWO3) based on a subset of 3 genes (CD3E IRF4 and ZAP70) whose abundance was independently associated with prognosis.<br>Classified tumours into 3 classes:<br>Immune rich (UWO3 ≤2)<br>Mixed (>2 and ≤2.5)<br>Immune desert (>2.5) | 5y-DFS and OS for the immune rich > mixed > immune deserts groups<br>OS: Immune rich > Mixed > Immune desert<br>Survival difference persisted regardless of primary treatment modality<br>Immune rich group had a superior DFS compared to mixed and immune desert group |

Abbreviations: *OPC* oropharyngeal carcinoma, *TIL* tumour infiltrating lymphocytes, *CPS* Combined Positive Score, *Trm T cells* tissue-resident memory T cells, *IHC* immunohistochemistry, *ITIC* intratumoural immune cells, *OS* overall survival, *DSS* disease-specific survival, *DFS* disease-free survival, *FFS* failure free survival, *TAMs* tumour-associated macrophages, *LRC* locoregional control, *LRF* locoregional failure, *MFS* metastases free survival, *DM* distant metastasis, *FU* follow-up, *MVA* multivariable analysis, *5y* 5-year, *RT/CETUX* radiation plus cetuximab, *RT/CIS* radiation plus cisplatin chemotherapy

## *Reappraising the Influence of EGFR Expression and Inhibition*

In a correlative study of locally advanced HNC enrolled in a phase III trial published in 2002, Ang et al. showed that epidermal growth factor receptor (EGFR) expression varied considerably among patients with locoregionally advanced head and neck cancer (LAHNC) and patients with EGFR overexpressing tumours had a reduced (LRC and OS, but no difference in distant control (DC) [32]. Subsequently, a phase III trial [33] (a.k.a. the "*Bonner Trial*") demonstrated that the addition of cetuximab, an anti-EGFR monoclonal antibody, to conventional radiation resulted in better LRC compared to radiotherapy alone. These studies were conducted without knowledge of tumour HPV status. Subsequently, an *ad hoc* retrospective analysis of the "*Bonner Trial*" by p16 status with modest sample size [34] showed that p16+ patients who received radiation with cetuximab (n = 41) had better LRC vs those without (n = 34). Surprisingly, there was no difference in LRC between p16-negative patients with (n = 43) vs without (n = 64) cetuximab, which is counterintuitive. Notably, the p16 and HPV concordance for this study was also lower than expected, which the authors attributed to possible tissue sample degradation over the ensuing years since the initiation of the trial.

It is now known that HPV+ OPC has a consistently low expression of EGFR [35, 36], and is more radio/chemo-sensitive. It is conceivable that the better outcomes with low EGFR expression might actually be a surrogate reflecting treatment results of HPV+ OPC. Nonetheless, the promising "*Bonner Trial*" results led to the design of three phase-III HPV+ OPC-specific de-intensification trials (RTOG 1016 [2], De-ESCALaTE [1], and TROG 12.01 [3]), with the hypothesis that cetuximab could replace cisplatin to achieve non-inferior tumour control with less severe toxicities. Unfortunately, the three trials showed uncharacteristically high locoregional

failure (LRF) rates with cetuximab. The two earlier studies (RTOG 1016 and De-ESCALaTE) also included T4 and N3 patients (without distinction in RTOG 1016, but restricted to none or mild smokers in De-ESCALaTE due to their putative low risk derived from the risk-stratification RPA analysis) who fared the worst following withholding of cisplatin; however this did not explain the full result since other stage subgroup also fared poorly. The TROG 12.01 trial [3] showed similar results while representing a more favourable population overall. It was designed later than the other two trials and therefore excluded T4 and N3 cases due to emerging data at the time. However, it also found inferior outcome due to both increased DM and LRF, and reduced failure free survival, on the cetuximab arm. These results raise the possibility that anti-EGFR therapy might interfere with radiosensitivity in HPV+ OPC [37]. In fact, Alsahafi et al. [38] studied HPV+ HNC cell lines and tumour tissues and found that EGFR downregulated HPV oncoprotein E6 expression and induced p53 activity in response to radiotherapy suggesting that inhibition of EGFR could reduce radiation-induced cell apoptosis. Clinical data regarding a potential detrimental effect of cetuximab has also emerged. The long-term outcome analysis of the RTOG 0522 trial [39] clearly showed a 15% increase in 5-year LRF rates (25% vs 10%) and double the risk of LRF (HR 2.04, 95% CI 1.08–3.85, $p = 0.028$) in p16+ OPC patients who received cetuximab in addition to cisplatin CRT. The NRG-HN004 trial [40] also showed reversed relationship in cetuximab vs durvalumab (anti-PD-L1 monoclonal antibody) on progression-free survival (PFS) between HPV+ and HPV-negative HNC.

In summary, evolving knowledge on EGFR expression and HPV+ OPC, and convincing data on increased LRF with cetuximab in combination with chemoradiation, together with translational evidence of cetuximab interference with radiation sensitivity in HPV+ OPC tumours and cell lines, suggest that it is time to sunset EGFR inhibition in combination with chemoradiation as a strategy to enhance LRC in the curative management of HPV+ OPC.

## *The Prognostic Importance of Imaging-detected Extranodal Extension*

The lymph node (LN) capsule comprises dense connective tissue stroma and collagenous fibres. It is a natural barrier that could prevent further tumour progression. Tumour penetration of the LN capsule is termed extranodal extension (ENE) and is believed to be a consequence of: (1) Faster tumour cell proliferation that outpaces the capacity of nodal capsular expansion, thereby *"breaching"* the LN capsule, (2) Factors within the tumour or tumour microenvironment that facilitate "transgression" of lymph nodal capsules (a hypothesis that may explain the presence of ENE in small LNs), and (3) A "neglected" tumour (intranodal tumour growth over an extended period resulting in a large tumour volume that "ruptures" the lymph node capsule). The first two causes probably implicate a more aggressive tumour phenotype. It has been reported that ENE+ OPC is associated with a higher nodal burden

(larger LN, higher number of LNs, higher N categories) [41–43], more frequent intranodal necrosis [44, 45], and higher FDG uptake on PET [46]. Numerous translational studies over the past two decades have also associated ENE with molecular biomarkers that indicate more aggressive phenotypes [47]. For example, podoplanin is a transmembrane glycoprotein expressed on the endothelial cells of lymphatic vessels [48] and can promote tumour invasion and metastasis [48, 49]. A recent study observed high perinodal podoplanin expression although no difference in intranodal expression [50]. Finally, investigators at the Danna-Farber Cancer Institute recently found an association of individual imaging features of ENE with baseline HPV circulating DNA (HPV-ctDNA) (see discussion later) [51].

The prognostic importance of ENE was recognized in non-viral related head and neck cancers, but has been under-appreciated in the more recently recognised HPV+ OPC due to conflicting results in earlier small surgical series which suggested a lack of prognostic impact from pathologic ENE (pENE) [52, 53]. However, this view has been dispelled in subsequent larger surgical series indicating strong prognostic value of pENE in OS [54–58], even in N1 disease [55, 59]. As an additional observation, the administration of cisplatin chemotherapy did not translate into better OS [54].

In HPV+ OPC patients treated with primary radiation (RT)/CRT), imaging-detected ENE (iENE) has emerged as one of the strongest baseline anatomic prognostic factors in HPV+ OPC, with an independent strong influence throughout all stage groups of the 8th edition TNM (TNM-8) [57, 60–63]. A meta-analysis showed that both pENE and iENE are prognostic, but iENE had an even numerically higher hazard ratio (HR) for OS than pENE (HR 2.64 vs 1.89) [57]. As mentioned below, this would be explained by the fact that iENE represents a more overt variant of ENE determined by imaging compared to pENE. The latter presumably identifies additional subclinical microscopic extension beyond the lymph node capsule that probably carries a less adverse prognosis due to the impact of contemporary treatments. A single institution study showed that iENE can identify a subgroup of Stage I (T1–2N1) HPV+ OPC patients with high risk of DM and death [61]. The prognostic importance of iENE was subsequently confirmed in a study comprising a cohort of HPV+ OPC that included all N categories and suggested categorizing iENE+ N1–N2 disease higher in the TNM stage classification [41]. In considering the potential prognostic importance, several deintensification trials have already excluded cases with some form of iENE in the clinical trial design phase. For example, NRG HN002 [64] and E3311 [65] trials both excluded those with "matted" lymph nodal masses. ORATOR [66] and ORATOR 2 trials [67] both excluded any sign of iENE for trial enrolment.

The prognostic importance of iENE is increased risk of DM with less impact on LRC. This is likely due to relatively radiosensitive nature of HPV+ OPC. Contemporary treatment delivers sufficient local treatment intensity and encompasses a sufficiently large radiotherapy volume could reliably address potential regional microscopic tumour foci, but unable to address tumour foci in

bloodstream as a result of iENE [41]. The increased risk of DM with iENE is understandable since tumour escaping the nodal capsule permits access to the bloodstream. Cisplatin, the most commonly used chemotherapy agent, is a potent radiosensitizer, but appears unable to fully negate the DM risk in ENE+ patients [41]. How to address increased risk of DM in iENE+ patients remains an active research area and will likely require newer systemic treatment strategies (e.g. see earlier discussion regarding "immune desert" phenotypic tumours). Presence of iENE could also impact the extent of elective radiotherapy neck volumes, especially when the extranodal disease invades neck muscles, thereby increasing the risk of microscopic tumour cell "migration" along muscle fibres beyond traditional clinical target volume (CTV) margin guidelines. For example, it has been recommended by Gregoire et al. as early as 2006 [68] that when ENE has invaded muscle, the CTV volume should encompass the entire muscle plane adjacent to the involved nodes.

The major challenge to adopting the iENE parameter in clinical practice and risk stratification is uncertainty of reliable iENE assessment, attributable to lack of consensus definition and insufficient awareness of the prognostic value of unequivocal evidence reported by radiologists. In contrast, uncertain declaration of findings may be either spurious or be of much less importance due to minimal disease presence. With respect to the latter, some might also dismiss the significance of iENE due to its inability to detect minimal pathological ENE (pENE) only visible through the pathologist's microscope. While the latter may be true, it is unlikely at this time for radiological imaging to be able detect this degree of minimal disease but it is also likely that contemporary radiotherapy with chemotherapy is capable of mitigating its presence; unfortunately, the same is not the case for overt and unequivocal radiologically evident disease that carries a high risk of failure, especially DM. To consolidate iENE definitions, an international expert panel was recently convened under the auspices of the Head Neck Cancer International Group (HNCIG) that undertook a Delphi process [69]. The panel agreed that iENE criteria should include any of the following: (1) Indistinct or irregular nodal margin or border, (2) Extension into perinodal fat, (3) Extension into adjacent structures (e.g. muscle, skin, salivary glands, and neurovascular bundle), and (4) Conglomerate/matted/coalescent nodes (all of which are considered to be synonymous). The expert radiologists concluded by agreeing to support the use of a standardized classification system and synoptic reporting in their routine clinical practice [69].

In summary, unequivocally declared iENE is a strong prognostic factor that can identify HPV+ OPC patients with higher risk of DM. iENE may manifest in various forms reflecting different degrees of nodal disease extension. Recommendations from radiologists for its definitions have been endorsed by 19 national head and neck research organizations, including clinical trials groups, representing 34 countries. More work, especially in the educational sphere, will be needed to improve the reliability of iENE assessment as practitioners become more familiar with its use in clinical trials and potentially in routine clinical practice.

## Dynamic (Post-treatment) Factors

### *Potential Importance of Tumour Volume Kinetics: Treatment Response*

It is well-known that most HPV+ OPCs are intrinsically chemo-/radio-sensitive, although heterogeneity exists. One of the surrogates for chemo-/radiosensitivity is *response to treatment*. Induction chemotherapy (IC) in HPV+ OPC to enhance local-regional and distant control is not commonly recommended in evidence-based guidelines due to the failure to demonstrate survival benefit in the first trials designed to compare it against concurrent CRT. In truth, such trials were not designed to address HPV+ OPC specifically at a time when its natural history was not completely appreciated (specifically the risk of DM in high-risk groups).

Several trials have now used response to IC as stratification factor to triage subsequent treatment, and generally >50% response at primary and the neck is considered *major partial response* (mPR), and together with those achieving a *complete radiological response* (CR) may be considered de-intensification candidates [70–73]. The E1308 trial demonstrated a positive correlation between responsiveness to IC and subsequent control using lower dose radiotherapy [71]. The OPTIMA trial (NCT02258659) [74] stratified patients as baseline low-risk ($\leq$T3 & $\leq$N1 & $\leq$10 PY) and high-risk (T4 or N2 or >10 PY), but also combined it with the response following 3 cycles of IC with carboplatin and nab-paclitaxel. They treated baseline low-risk and mPR/CR patients with low-dose RT alone to 50 Gy, baseline low-risk with 30–50% PR, together with baseline high-risk achieving mPR or CR, using low dose CRT; the remainder received standard CRT. As discussed below, the OPTIMA trial also used a post-IC radiotherapy volume reduction based on initial work reported by the same group with a view to toxicity reduction as well as tissue protection advantages [75]. The 2-year OS/DFS was 100%/95% for baseline low-risk, and 97%/94% for baseline high-risk patients.

The iChoice-01 trial (NCT04012502) treated HPV+ OPC patients who achieved mPR/CR at both primary and neck after two cycles of docetaxel-cisplatin-fluorouracil (TPF) IC with reduced dose RT alone (60 Gy in 30 fractions). Only 1 patient (T1N3, with iENE+) in the de-intensification arm experienced regional failure and lung DM 25.3 months after initial treatment. Notably all 8 patients who experienced recurrence in the study (1 in the deintensification and 7 in the standard cisplatin chemoradiation arm) had presence of iENE.

As already mentioned, beside dose reduction, another usage of treatment response to IC as a dynamic biomarker is to select patients who might be suitable for volume reduction, which is emerging as increasingly important in the immunotherapy era where sparing of healthy regional nodal drainage areas may be a critical step for T cell priming to permit the effectiveness of PD-1 blockade [76, 77]. Villaflor et al. [75] conducted a phase II response-adapted volume de-escalation trial (RAVD, NCT01133678) that enrolled 93 locally advanced HNC patients (51 were HPV+ OPC), where patients who achieved mPR/CR following two cycles of

IC (paclitaxel, 5-fluorouracil, hydroxyurea) received RT to gross tumour only, with 1.5 cm expansion without elective volumes. They reported 2-year PFS of 93% for HPV+ OPC who achieved mPR/CR, suggesting that elimination of elective nodal coverage did not appear to compromise oncologic outcomes with the added advantage of toxicity reduction and conceivable benefit of preserving healthy lymphocytes in normal draining lymph nodes. This strategy has been further extended to the design of the OPTIMA and OPTIMA II trials. For example, the OPTIMA II trial (NCT03107182) [72] incorporated nivolumab (anti-PD-1 antibody) at induction and adjuvant phase with a similar post-induction risk stratification and RT/CRT volume de-escalation for the subsequent treatment as employed in the OPTIMA trial and originally proposed by Villaflor et al. [75]. A preliminary report at ASCO 2022 showed excellent 2-year OS and PFS for both low-risk and high-risk patients.

Finally, intra-treatment response two-week into radiation based on FDG PET [78] or hypoxia imaging [79] has also demonstrated promising outcomes.

In summary, induction chemotherapy in HPV+ OPC appears to have untapped potential despite the absence of obvious benefit for head and neck cancer in general in previous clinical trials. Reports suggest that 50–70% of HPV+ OPC patients may achieve mPR or CR after IC [70–73]. It is conceivable that this could follow the path set by the other well established viral-induced pharyngeal cancer with high risk of DM in certain subgroups (i.e. Epstein-Barr virus-related nasopharyngeal cancer) where IC has set a new standard of care due to its impact on reducing DM and ultimately improving OS. Therefore, response to IC could serve as a biomarker for intrinsic chemo/radiosensitivity. Together with baseline tumour factors, treatment response could influence subsequent treatment strategies that might include radiation treatment volume and dose reduction. Furthermore, it also provides opportunities to incorporate other systemic agents to reduce DM.

## *The Prognostic Value of HPV-ctDNA*

Liquid biopsy is a non-invasive diagnostic approach to detect, characterize, and monitor tumour burden prior to and following treatment using one or more body liquids, including blood, urine, saliva, feces, pleural effusions, ascites, and cerebrospinal fluid [80–82]. Detection of DNA in body fluid is referred to under the rubric of circulating cell-free DNA (cfDNA), although it does not always emanate from tumour cells. Circulating tumour DNA (ctDNA) is potentially only a fraction of cfDNA that originates from tumour cells. It arises as the product of three activities: apoptosis, necrosis, or active secretion. It is also important to differentiate the two (ctDNA from cfDNA) [83].

HPV+ tumours can shed HPV DNA into body fluid to form HPV circulating tumour DNA (HPV-ctDNA). In the context of patients with known diagnosis of HPV+ OPC, detection of HPV DNA in the plasma is almost exclusively derived from tumor (i.e. ctDNA). Plasma HPV-ctDNA can be found in approximately 90% of HPV+ OPC patients at the time of diagnosis with a modest sensitivity (ranging

between 60% and 90%) and a very high specificity (approaching 100%) for detection of HPV+ cancers at diagnosis and follow-up [84–89].

Conflicting data exist regarding the role of HPV-ctDNA at baseline for risk stratification (Table 15.2). Chera et al. [86] showed some contrasting correlations of pretreatment HPV-ctDNA copy number and elements within the TNM Stage Classification. Thus, for the primary T categories: T2 tumours had higher HPV-ctDNA vs T0/T1 tumours, but T3/T4 tumours had significantly lower baseline HPV-ctDNA levels; similarly, TNM-7 N2a/N2b patients had higher HPV-ctDNA levels vs N0/N1 disease, but N2c had a borderline lower HPV-ctDNA vs N2a/N2b. However, it is possible that it does not truly reflect tumour burden since only the

**Table 15.2** Selected studies on role of HPV-ctDNA in risk stratification and disease surveillance in HPV+ OPC

| Author, Year | Study description | Findings |
|---|---|---|
| Adrian, 2023 [97] | n = 136<br>p16+ve OPC treated with CRT from the ARTSCAN III RCT (NCT01969877)<br>Blood samples collected pre- and post- CRT<br>Viral load of HPV16-ctDNA using qPCR<br>Endpoint: prognostic value of HPV16-ctDNA pre- and post-CRT<br>Median FU: 2.8 y | Disease burden (GTV-T and GTV-N) correlated with baseline HPV16-ctDNA levels ($p < 0.001$)<br>Lower baseline HPV16-ctDNA associated with better PFS ($p = 0.01$), OS ($p = 0.01$) but not LC ($p = 0.12$)<br>AUC- HPV16-ctDNA (combined pre and post levels) remained significant prognostic for PFS |
| Califano, 2023 [98] | n = 233<br>Cohort study (patients from DE-ESCALATE HPV trial) of low-risk HPV+ OPC patients<br>Presence of HPV16-ctDNA in plasma and salivary rinse tested by qPRC tested pre-tx, 3 and 12 m post-tx<br>Endpoint: lead time HPV16-ctDNA detection to recurrence<br>Median FU 760 days (2.08 y) | Plasma HPV16-ctDNA assay sensitivity 35%; specificity 98%<br>Salivary rinse HPV16-ctDNA assay sensitivity 45%; specificity 87%<br>Combined plasma and salivary rinse HPV16-ctDNA assay sensitivity 65% and specificity 87%<br>Lead time of positive test to event/recurrence: median 19 days (0–536 days) and mean 122 days (SD 169.8 days) |
| Cao, 2022 [93] | n = 34<br>Stage III (TNM-8) p16+ OPC from a phase II trial (NCT02031250)<br>Blood samples collected at baseline, during CRT week 2,4, 7 and at follow up 3, 6, and 12 m post-tx<br>Endpoints: kinetics of HPV-ctDNA with tumour progression post CRT AND compare predictive value of HPV-ctDNA to imaging biomarkers of MRI and FDG PET<br>Mean FU: 28 m | Higher pre-tx HPV-ctDNA level, higher risk of tumour progression (HR = 1.06 [95% CI of 1.01–1.12], $p < 0.03$)<br>An early increase in HPV-ctDNA at week 2, better PFS (HR 0.11, [90.01–0.95]).<br>Clearance of HPV-ctDNA at weeks 4 and 7 were not predictive of PFS<br>Smoking status did not predict PFS ($p > 0.9$)<br>Pre-tx HPV-ctDNA levels were correlated with nodal GTV, but not with primary tumours |

(continued)

**Table 15.2** (continued)

| Author, Year | Study description | Findings |
|---|---|---|
| Chera, 2019 [86] | n = 103<br>p16+ OPC, RT/CRT in 2016–2018<br>Prospective phase II study (NCT0316182)<br>Blood specimens collected at baseline, weekly during RT/CRT and FU visits (q2–4m at years 1–2, q6m at years 3–5<br>Quantify HPV-ctDNA dPCR assay (16/18/31/33/35): multianalyte digital PCR assays<br>Endpoint: identify profile of HPV-ctDNA clearance kinetics post CRT<br>Median FU: 16.5 m | Baseline HPV16-ctDNA high specificity (97%) and sensitivity (89%)<br>Baseline HPV16-ctDNA copy number correlated with disease burden and HPV integration status<br>Higher HPV16-ctDNA in T2 vs T0/T1 but lower in T3/T4 tumours<br>Higher HPV16-ctDNA in TNM-7 N2a/N2b vs N0/N1, but lower in N2c vs N2a/N2b<br>Lower HPV16-ctDNA in T4 tumour or >10 PY smoking<br>Lower baseline HPV16-ctDNA, higher likelihood of HPV integration into the somatic genome<br>Rate of HPV16-ctDNA clearance (>95% clearance of baseline levels by week 4) correlates with high RT/CRT sensitivity and lower rate of regional failure<br>A favourable HPV16-ctDNA clearance profile: high baseline level (>200 copies/mL) with rapid clearance (>95% clearance by week 4). |
| Chera, 2020 [90] | n = 115 [CRT 2016–2018]<br>Prospective study p16+ve OPC treated with CRT enrolled in 3 trials (NCT0316182; NCT02281955; NCT03077243)<br>Blood samples collected every 6–9 m at FU for up to 5y<br>Plasma HPV-ctDNA detected using multianalyte digital PCR<br>Endpoint: NPV and PPV of HPV-ctDNA for disease recurrence<br>Median FU: 23 m | 16/115 (14%) had 2 consecutive HPV-ctDNA+ at FU<br>Two consecutive HPV-ctDNA+ tests for disease recurrence:<br>Sensitivity: 100%; specificity: 99%<br>PPV: 94% (95% CI 70–99%); NPV: 100% (96–100%)<br>Patients with 2 consecutive HPV-ctDNA+ tests:<br>2y RFS: 5% (vs 100% in the remaining)<br>2y LRC: 65% (vs 100% in the remaining)<br>2y DMFS: 21% (vs 100% in the remaining)<br>Median lead time between HPV-ctDNA+ and biopsy proven recurrence was 3.9 m (range 0.37–12.9 m) |

(continued)

**Table 15.2** (continued)

| Author, Year | Study description | Findings |
|---|---|---|
| Ferrandino, 2023 [99] | n = 399<br>163 diagnostic cohort<br>290 surveillance cohort<br>Retrospective observational cohort study<br>Endpoint: per test sensitivity, specificity, positive predictive value, negative predictive value for TTMV-HPV-DNA<br>Median follow up (surveillance cohort) was 40.5 (17–67.5) months | Diagnostic cohort: TTMV-HPV-DNA for diagnosis<br>Sensitivity 91.5% [85.8–95.4%]<br>Specificity 100% [71.5–100%]<br>Surveillance cohort: TTMV-HPV for recurrence detection<br>Sensitivity 88.4% [74.9–96.1%]<br>Specificity 100% [99.3–100%]<br>PPV: 100% [90.7–100%]<br>NPV: 99.1% [97.9–99.7%]<br>Median lead time to pathological confirmed recurrence: 47 (0–507) days |
| Hanna, 2023 [100] | n = 543, p16+ OPC<br>Retrospective study<br>Plasma TTMV-HPV-DNA test at 0–3 vs 3–6 vs 6–12 vs 12–24 vs >24 m post-tx<br>Endpoint: NPV of TTMV-HPV-DNA for recurrence<br>Median FU 27.9 m | 236 (44%) had ≥3 tests during surveillance<br>55 (10%) had recurrences<br>Per test sensitivity = 92.5% [95% CI 87.5–97.5]<br>Per patient sensitivity = 87.3% [95% CI 79.1–95.5]<br>NPV per test = 99.4% [95% CI 98.9–99.8]<br>NPV per patient = 98.4% [95% CI 97.3–99.5] |
| Rettig, 2022 [51] | n = 110 [2019–2022]<br>Cross sectional study p16+ OPC<br>Pre-treatment TTMV-HPV-DNA from 5 genotypes (16, 18, 31, 33, and 35) using dPCR<br>Endpoint: clinicopathologic characteristics | Most detectable TTMV-HPV-DNA genotype was HPV16 (88%)<br>Detectability of TTMV-HPV-DNA increased with higher cN categories (95% detectable in N1–N3; undetectable TTMV HPV-DNA 58% in N0 stage)<br>iENE+ patient had a higher TTMVHPV-DNA scores (median 1438 vs 281) although it was not associated with TTMVHPV-DNA detectability<br>MVA showed higher cN categories and nodal SUV strongly associated with TTMV-HPV-DNA score |

Abbreviation: *OPC* oropharyngeal carcinoma, *RT* radiation, *CRT* chemoradiation, *FU* follow-up, *m* months, *y* years, *RCT* randomized control trial, *Tx* treatment, *DFS* disease-free survival, *OS* overall survival, *LC* local control, q2–4m every 2–4 months, *GTV-T* primary gross tumour volume, *HPV-ctDNA* HPV circulating tumour DNA, *TTMV* tumour tissue modified viral, *TNM-7* the 7th edition TNM classification, *PY* pack-year, *NPV* negative predictive value, *PPV* positive predictive value, *HNSCC* head and neck squamous cell carcinoma, *iENE* imaging-detected extranodal extension

episomal form of the virus that sheds into blood can circulate and be detected by the current assays: the integrated form seems not to circulate freely. Indeed, they further evaluated HPV structure in the tumours and found that a significantly higher proportion of patients with low HPV-ctDNA copy number (≤5 copies/haploid genome) had higher HPV integration resulting in lower release of HPV-ctDNA. Patients with high pretreatment HPV-ctDNA levels (>200 copies/mL plasma) were most likely to have tumours with high copy and episomal HPV, which correlates with favourable prognosis. In contrast, Rettig et al. [51] showed a positive association of HPV-ctDNA with N categories: the higher the median tumour-tissue-modified viral (TTMV) HPV DNA, the higher the N categories. In addition, Rettig et al. also showed a correlation between HPV-ctDNA and extranodal extension (ENE) which is not surprising given our earlier statement that ENE+ OPC is associated with a higher nodal burden (larger LN, higher number of LNs, higher N categories) as baseline descriptors of anatomic disease burden [51] and that ENE+ disease is associated with biological parameters associated with increased aggressiveness [47, 50].

Like circulating EBV DNA in nasopharyngeal carcinoma, clearance of HPV-ctDNA is emerging as a useful biomarker for disease surveillance, and the presence of post-treatment HPV-ctDNA is a diagnostic biomarker for molecular residual disease (MRD). Chera et al. showed that patients with detectable HPV-ctDNA in two consecutive plasma samples tested during disease surveillance had much higher risk of disease relapse compared to those with undetectable HPV-ctDNA at all subsequent times [90]. Early detection could prompt earlier initiation of salvage therapy. Haring et al. [91] also found a strong correlation of change of plasma HPV-ctDNA level and radiologic treatment response, and HPV-ctDNA-detected molecular residual disease (MRD) was observed an average of 70 days (range 35–166) earlier than conventional imaging-detected residual disease. Berger et al. [92] also showed that tumour tissue modified viral HPV DNA (TTMV-HPV-DNA) was the first indication of recurrence in 93% of HPV+ recurrent cases, predating identification of recurrence via routine clinical and imaging examinations.

Interestingly, increase of HPV-ctDNA is not always an adverse sign. There appears to frequently be a spike of HPV-ctDNA earlier in a course of radiotherapy, likely related to lysis of tumour cells by radiation, which might herald better outcomes. Cao et al. [93] showed a positive association with higher PFS with a low pretreatment HPV-ctDNA and an early increase in HPV-ctDNA at week 2 compared with baseline. However, the sample size was very small.

In summary, the role of baseline HPV-ctDNA remains uncertain but has promising appeal from early observations. It may not truly reflect tumour burden and therefore has limitations if it is to compliment or substitute for staging at baseline in the future, since only the episomal form sheds into the blood and can circulate while the integrated form seems to be more frequently expressed in the T4 tumours and does not circulate. However, the dynamic changes or clearance of HPV-ctDNA level after treatment is a strong prognostic factor that can identify clinically occult disease that bear MRD status. Whether early detection and early intervention for MRD translate to clinical benefit remains to be evaluated. In addition, standardized testing algorithms, increasing sensitivity with reduced cost, and efforts to make it commercially

available and economically feasible for patients everywhere are paramount for adoption of HPV-ctDNA as a surveillance tool [82]. One will also need to remain cautious about false negative levels if the problem discussed concerning the non-circulating integrated form translates over to the surveillance context. In the end, well designed trials will be needed to address HPV-ctDNA identified MRD.

## Conclusion

Prognostic risk stratification requires careful derivation. The role of smoking on HPV+ OPC patients' survival is evident, but the underlying mechanisms are enigmatic and diverse: its "impact" is significantly confounded by competing risks, often non-oncologic and unrelated to the index primary. There is no obvious prognostic impact in surgically treated cases, no apparent association with increased mutational burden or recurrent oncogenic mutations, but a possible association with an "acquired" immune desert TME phenotype may exist. Immune profiles to differentiate immune-rich vs immune-desert subsets provide potential new therapeutic opportunities. iENE is an under-emphasised anatomic biomarker that carries a significant risk of predicting DM and may play an important role in baseline risk stratification and might further refine the current cN classification within the TNM classification for HPV+ OPC. Liquid biopsy assessments may contribute to baseline assessment, and even staging, but require significant additional refinement, clarification and assay development, as well as harmonisation. In the post-treatment arena, anatomical and biological (e.g. liquid biopsy) response to treatment seems relevant to treatment modification and surveillance while also bearing in mind the caveats concerning the robustness of the assay.

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# Chapter 16
# Immunotherapy and Novel Agents in Locoregionally Advanced HNSCC


**Séverine Carlier and Jean-Pascal Machiels**

## Abbreviations

| | |
|---|---|
| CRT | Chemoradiation therapy |
| ctDNA | Circulating tumour DNA |
| DDR | DNA damage response |
| DFS | Disease-free survival |
| DSBs | Double-strand breaks |
| EFS | Event-free survival |
| EGFR | Epidermal Growth Factor Receptor |
| HNSCC | Squamous cell carcinoma of the head and neck |
| IAP | Inhibitor of apoptosis proteins |
| IFN-γ | Interferon gamma |
| IMRT | Intensity-modulated radiation therapy |
| LA | Locally advanced |
| LRC | Loco-regional control |
| mAbs | Monoclonal antibodies |
| MRD | Minimum residual disease |
| OR | Odds ratio |
| ORR | Objective response rate |
| OS | Overall survival |

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| | |
|---|---|
| PARP | Poly(ADP-ribose) polymerase |
| PD(L)-1 | Programmed cell death protein-(ligand) 1 |
| PFS | Progression free survival |
| R/M | Recurrent and/or metastatic |
| ROS | Reactive oxygen species |
| SMAC | Second Mitochondria-Derived Activator of Caspases |
| SSBs | Single-strand breaks |
| TMB | Tumor mutational burden |
| TNF | Tumor necrosis factor |

## Introduction

Curative treatments of squamous cell carcinoma of the head and neck (HNSCC) typically) involve single or combined approaches such as surgery and/or (chemo) radiation, depending on disease stage, location, and expected functional outcomes [1]. These treatments yield survival rates of 80% and 50% at 5 years for early and advanced stages, respectively. Approximately, two thirds of patients with HNSCC present with locally advanced (LA) disease at diagnosis. To improve these relatively low survival rates, several strategies are being investigated.

So far, anti-programmed cell death protein-1 (anti-PD-1) and anti-programmed death-ligand 1 (PD-L1) monoclonal antibodies did not succeed to improve outcome in LA-HNSCC, unlike their proven benefit in recurrent or metastatic disease [2]. In this chapter, we will discuss how to better integrate PD-1/PD-L1 inhibitors into the curative treatment of HNSCC, with the lessons learned from previous trials. Then, we will explore four different novel therapy classes (non-immunological) currently under investigation in LA HNSCC.

## Anti-PD-1/PD-L1 Monoclonal Antibodies (mAbs) in Combination with Curative-Intent Primary (Chemo) radiation

Anti-PD-1/PD-(L)1 mAbs in combination with (chemo)radiation have been investigated in several indications: (i) as a de-escalation strategy in good prognosis patients (e.g., stage I/II p16-positive oropharyngeal cancer), (ii) to replace chemotherapy in cisplatin-unfit patients, and (iii) as treatment intensification in combination with chemoradiation in poor prognosis patients (e.g., p16-negative LA-HNSCC) and stage III p16-positive oropharyngeal cancer). Until now, trials that combined PD-(L)1 inhibitors with curative-intent therapy did not meet their primary endpoints. These trials are summarized in Table 16.1. Following these results, several strategies are currently investigated to better integrate immunotherapy in the

**Table 16.1** Phase 3 trials investigating anti-PD-(L)1 inhibitors in locally advanced head and neck squamous cell carcinoma

| Trial (Reference) | N | Study description | Outcome |
|---|---|---|---|
| JAVELIN 100 [3] | 697 | Chemoradiation ± avelumab | PFS<br>HR 1.21 (95% CI: 0.93, 1.57) |
| KEYNOTE 412 [4] | 804 | Chemoradiation ± pembrolizumab | EFS<br>HR 0.83 (95% CI: 0.68, 1.03) |
| REACH (fit) [43] | 430 | Chemoradiation vs RT + cetuximab + avelumab | PFS<br>HR 1.27 (95% CI: 0.83, 1.93) |
| REACH (unfit) [43] | 277 | RT + cetuximab vs RT + cetuximab + avelumab | PFS<br>HR 0.84 (95% CI: 0.62, 1.15) |
| NRG-HN004 (unfit) [44] | 186 | RT + cetuximab vs RT + durvalumab | 2-year PFS rate<br>62% vs 51% |
| IMVoke010 [9] | 406 | Adjuvant atezolizumab vs placebo (post definitive therapy) | EFS<br>HR: 0.94 (95% CI: 0.70, 1.26). |

*PFS* Progression-free survival, *EFS* Event-free survival, *RT* Radiotherapy, *HR* Hazard ratio, *CI* Confidence interval

multimodal treatment of LA-HNSCC such as better patient selection, optimization of treatment sequence, and the lymph node irradiation field.

## *Patient Selection*

Not all patients seem to benefit equivalently from immunotherapy when considering subgroup analyses. Several studies tend to show a benefit in tumors with a PD-L1 score >0. First, JAVELIN 100 compared high dose cisplatin chemoradiation combined with avelumab or placebo. The trial did not meet its primary endpoint: median progression-free survival (PFS) was 16.9 months in the avelumab arm and not reached in the control arm ($p = 0.92$). Subgroup analysis suggested that PD-L1 expressing tumors might benefit from the addition of avelumab, although this analysis was impaired by the low number of patients [3]. Second, KEYNOTE-412 showed that pembrolizumab plus chemoradiotherapy did not significantly improve event-free survival (EFS) compared with chemoradiotherapy alone [4]. In the PD-L1 ≥20 subgroup, median EFS was not reached in either arm; at 36 months, 66.7% of patients in the pembrolizumab arm were event-free compared with 57.2% in the control arm (HR = 0.73; 95% CI = 0.49–1.06). In this study, subgroup analyses suggested a higher benefit in HPV-negative tumors (HR = 0.83; 95% CI = 0.66–1.05), stage IV disease (HR = 0.81; 95% CI = 0.64–1.03), and hypopharynx cancer (HR = 0.70; 95% CI = 0.43–1.15) as well as an improved control of

distant metastases with the addition of pembrolizumab (12.9% vs 16.7% in the pembrolizumab and control arms, respectively).

Ultimately, selecting PD-L1 expressing tumors appears to be a promising option for integrating immune checkpoint inhibitors (ICI) with multimodal curative treatment. Additionally, it might be beneficial to consider HPV-negative tumors, disease location, and stage, as these factors are associated with an increased risk of distant metastases. Furthermore, exploring other immune biomarkers such as tumor mutational burden (TMB), CD8+ T cells infiltration, and interferon gamma (IFN-γ) levels could provide further insights into patient response and guide treatment decisions.

On the other hand, we could also consider a negative selection approach. In all these trials, it is evident that the control arm exhibited significant efficacy, with approximately 50% of patients achieving cure with chemoradiation alone. Identifying this subgroup of patients who may not require treatment intensification with immunotherapy, as they are likely already cured by the radio-chemotherapy, is crucial for optimizing therapeutic strategies. Circulating tumor DNA (ctDNA) has shown promising results in other cancer types to detect minimal residual disease (MRD) after a curative-intent treatment. In the phase III IMvigor010 adjuvant study for muscle invasive urothelial carcinoma [5], patients who were tested positive for ctDNA after surgery demonstrated improved disease-free survival (DFS) and overall survival (OS) in the atezolizumab arm compared to the observation arm (DFS HR = 0.58 (95% CI: 0.43–0.79); p = 0.0024, OS HR = 0.59 (95% CI: 0.41–0.86)). No differences in DFS or OS between treatment arms were noted for patients who were tested negative for ctDNA after surgery. Regarding HNSCC, a tumor-agnostic plasma ctDNA assay with a 26-gene panel has been developed and tested to detect MRD in unselected LA-HNSCC (for both HPV positive and negative tumors) [6]. This study met its primary endpoint with a 2-year PFS of 23.5% (95% CI 10–55%) and 86.6% (95% CI 73.4–100%) in MRD-positive and MRD-negative patients, respectively ($p < 0.05$). Investigating whether ctDNA can serve as a biomarker to better select patients who would benefit from intensified therapies, such as immunotherapy, is crucial for future studies.

## *Treatment Sequence*

Another important aspect regarding the use of immunotherapy for LA-SCCHN seems to be its place in the treatment sequence. For now, it remains unclear what is the best timing to administer the anti-PD-(L)1 inhibitor: concomitant or sequential, adjuvant or neo-adjuvant.

### Concomitant

The JAVELIN 100 and the KEYNOTE-412 trials [3, 4] investigated concomitant and adjuvant anti-PD-(L)1 with cisplatin-based chemoradiation and failed to demonstrate a benefit for the immunotherapy arm. In PembroRad (GORTEC 2015-01)

[7], patients with LA-HNSCC unfit to receive high-dose cisplatin were randomized between radiotherapy (RT) with cetuximab and RT with pembrolizumab. Pembrolizumab was given only during RT and not as adjuvant therapy. Locoregional control (LRC), PFS and OS were similar between the two groups. Long-term analysis of these trials will be important, but these studies strongly suggest that there are little benefits (if any) to administer anti-PD-(L)1 concomitantly to (chemo) radiation.

### Adjuvant

A small randomized phase II trial evaluated fixed-dose concurrent versus sequential adjuvant pembrolizumab added to standard chemoradiation (CRT) in LA-HNSCC [8]. Sequential pembrolizumab conferred numerically higher PFS rates compared with concurrent pembrolizumab at 1 year (89% vs. 82%), 2 year (89% vs. 78%) and 3 year (84% vs. 74%), as well as higher OS rates (1-year, 95% vs. 82%; 2-year, 95% vs. 78%; 3-year, 90% vs. 74%). Median PFS and OS were not reached in both groups. This study suggested that sequential treatment may be preferable to concurrent treatment in testing immunoradiotherapy treatment strategies. Several Phase III studies are ongoing to determine whether sequential adjuvant immunotherapy after definitive local therapy can benefit patients with locally advanced HNSCC. The Phase III IMvoke010 trial (NCT03452137) was based on the same rationale than the PACIFIC trial in non-small cell lung cancer [9]. Patients with LA-HNSCC underwent definitive local therapy, which included CRT or surgery followed by RT or CRT. Subsequently, patients were randomized to receive adjuvant atezolizumab or a placebo. Unfortunately, this trial did not meet its primary endpoint as reported recently: 2-year EFS rate was 67.4% in the atezolizumab arm and 63.8% in the control arm (HR: 0.94 (0.70, 1.26), p = 0.68).

The ongoing NCT0811015 trial is another phase III study in patients with intermediate-risk HPV-associated oropharyngeal cancer, also investigating adjuvant immunotherapy. Following standard CRT, patients are randomized to receive adjuvant nivolumab or observation.

## *Neoadjuvant*

The concept of administrating immunotherapy as neoadjuvant therapy has been investigated in several cancers. In stage IIIB and IVC melanoma, neoadjuvant pembrolizumab followed by adjuvant treatment has already demonstrated a benefit in terms of EFS compared to a strictly adjuvant regimen [10]: 2-year EFS was 72% (95% CI, 64–80) in the neoadjuvant-adjuvant group and 49% (95% CI, 41–59) in the adjuvant-only group. So far, ICIs in the neoadjuvant setting in LA-HNSCC have shown promising results (high pathological response rate) in window of opportunity trials. Table 16.2 summarizes the various window of opportunity studies that have

**Table 16.2** Window of opportunity studies with immunotherapy in locally advanced squamous cell carcinoma of the head and neck (not exhaustive)

| Trial (Author) | N (Disease type) | Agent | ORR | Pathological response | Surgery delay |
|---|---|---|---|---|---|
| Ferris [45] | 26 HPV-positive (24 OPC) 26 HPV-negative | Nivolumab (2 cycles every 2 weeks) | HPV-positive: 12% HPV-negative: 8.3% | HPV-positive: 23.5% HPV-negative: 5.9% (<50% residual tumor cells) | No |
| Knochelmann [46] | 12 Oral cavity | Nivolumab (3 cycles every 2 weeks) | 33% (PD = 33%) | | No |
| Wise-Drapper [47] | 92 HNSCC | Pembrolizumab (one cycle) | NA | 39% (<20% residual tumor cells) *Better outcome for patients with response (1-year DFS 93 vs 72%)* | No |
| Uppaluri [48] | 36 HPV-negative | Pembrolizumab (one cycle) | 0% | 22% pTR-2 (>50% debris, necrosis, giant cells) | No |
| Oliveira [49] | 30 HPV-negative | Pembrolizumab (2 cycles every 3 weeks) | 19% (PD = 24%) | 44% pTR2 (with 16% < 10% viable tumor cells) | No |
| Ferrarotto [50] | 25 p16-positive OPC 4 p16-negative OPC | Durvalumab ± tremelimumab | 48% (similar in both arms) | 7% in both arms (<10% viable tumor cells) | 10% |
| Vos [51] | 32 HNSCC | Nivolumab (n = 6) Nivolumab + ipilimumab (n = 26) | NA But *no correlation with pathological response* | 17% (<10% residual tumor cells) 35% (<10% residual tumor cells) | No |

*HPV* Human Papilloma Virus, *HNSCC* Head and Neck Squamous Cell Carcinoma, *OPC* Oropharyngeal Cancer, *NA* Not available, *PD* Progressive disease, *DFS* Disease-free survival, *ORR* Objective Response Rate

investigated neoadjuvant immunotherapy treatment for LA-HNSCC. Following these encouraging results, the randomized, open-label, phase 3 KEYNOTE-689 trial (NCT03765918) is evaluating the efficacy and safety of pembrolizumab as neoadjuvant and adjuvant therapy, in the context of surgery followed by adjuvant (chemo)radiation, in patients with previously untreated, resectable locally advanced HNSCC [11].

The administration of anti-PD-(L1) inhibitors in monotherapy in the neoadjuvant setting may expose some patients to ineffective therapy and delay the curative-intent surgery (for example, due to possible immune-mediated toxicity or rapid disease progression caused by the known slower mechanism of action of immunotherapy compared to chemotherapy). Addition of polychemotherapy to anti-PD-(L)1 inhibitors may induce tumor shrinkage in a high proportion of treated patients thus allowing around 3 months of exposition to anti-PD-(L)1 inhibitor in the induction setting. This approach has been proved successful in non-small cell lung cancer [12, 13]. Several phase 2 trials have demonstrated the feasibility of combining platin/taxanes/anti-PD-(L)1 as neoadjuvant therapy in LA-HNSCC [14]. No phase 3 trial have been performed yet.

#### Regional Lymph Node Radiotherapy

Another possible explanation for the lack of benefit from ICI treatments in combination with chemoradiotherapy may be the large irradiation field to regional lymph nodes that might neutralize immune competent cells. To circumvent that possibility, the REWRITE trial (NCT03726775) is investigating the activity of durvalumab in combination with less extensive nodal radiation therapy (irradiation of adjacent lymph nodes only). The primary endpoint of this trial is the rate of relapse in non-irradiated regional lymph nodes in a highly selected population (T1–T4 with clinical status N0–N1 or N2a–N2b non-palpable).

## Novel (Non-immunological) Agents

### *SMAC Mimetics*

Second mitochondria-derived activator of caspases (SMAC) mimetics, a group of small-molecule compounds that imitate the function of the SMAC protein, enhancing caspase activation and consequently apoptosis by blocking inhibitor of apoptosis proteins (IAP). These agents hold promise in potentiating the efficacy of radiotherapy (see also Chap. 6 of this THNO issue). Their role in sensitizing tumors to radiation is mediated through caspase activation and various pathways involving tumor necrosis factor (TNF), IFN-γ, and CD8+ T cells [15].

**Table 16.3** Ongoing phase 2/3 randomized trials with xevinapant in locally advanced squamous cell carcinoma of the head and neck

| Trial (Reference) | Phase | N | Study description |
|---|---|---|---|
| TrilynX (NCT04459715) [52] | III | 700 | Chemoradiation ± xevinapant |
| XRAY VISION (NCT05386550) [53] | III | 700 | Post-operative radiotherapy ± xevinapant for resected LA-HNSCC non-eligible for cisplatin |
| EORTC 2120 (Ravina) (NCT05724602) [54] | II | 230 | Radiotherapy ± xevinapant in elderly patient (>70 years) |
| GORTEC (NCT05930938) [55] | III | 538 | Cetuximab with radiotherapy ± xevinapant cisplatin-unfit patients |

*LA-HNSCC* Locally advanced squamous cell carcinoma

Xevinapant, an oral first-in-class IAP inhibitor, was investigated in a phase 2 double-blind, randomized study [16, 17]. This trial met its primary end point of improving locoregional control at 18 months (Odds ratio (OR), 2.74; 95% CI, 1.15–6.53; p = 0.0232) and PFS (HR, 0.33; 95% CI, 0.17–0.67; P = 0.0019) with the experimental regimen (oral xevinapant with standard high-dose cisplatin chemoradiotherapy) versus control therapy (placebo with standard high-dose cisplatin chemoradiotherapy). Median OS was not reached with xevinapant and was 36.1 months in the placebo group. The 5-year OS rate was 53% (95% CI, 37–66%) versus 28% (95% CI, 15–42%) in the xevinapant and control groups, respectively [18].

Based on these results, several phase II/III studies are underway to better determine the role of xevinapant in curable HNSCC. These trials are described in Table 16.3. Other SMAC mimetics such as tolinapant (ASTX660) and birinapant (TL32711) are currently being evaluated in combination with radiotherapy in phase 1 clinical trials.

## *Drugs Targeting the DNA Damage Response (DDR) Pathways*

Ionizing radiation induces cell death in cancer cells through multiple mechanisms, including the generation of reactive oxygen species (ROS), DNA damage, and stress responses within mitochondria and the endoplasmic reticulum [19–21]. ROS, for example, causes DNA damage by causing both single-strand breaks (SSBs) and double-strand breaks (DSBs) [22]. This DNA damage initiates various signaling pathways, leading to cell cycle arrest and activation of the DNA damage response (DDR) pathways. This could result in treatment resistance [23]. Therefore, targeting the DDR signaling pathways to overcome tumor resistance to radiotherapy and/or chemotherapy is an attractive approach. The DDR pathways include the activation of several proteins [24], such as ATM, ATR, and DNA-PK kinases. These kinases, in turn, activate downstream proteins like CHK1, CHK2, and p53. They are recruited to and activated at sites of DNA damage by specific sensor protein complexes—MRE11/RAD50/NBS1 for ATM, RPA/ATRIP for ATR, and KU70–KU80/86 (XRCC6/XRCC5) for DNA-PKcs. Following activation, ATM, ATR, and DNA-PKcs

phosphorylate numerous substrates that overlap partially to facilitate effective and precise DNA repair, as well as to synchronize DNA repair with other DNA metabolic processes (e.g., transcription, replication, and mitosis). Additionally, poly(ADP-ribose) polymerase (PARP) plays a critical role in repairing both SSBs and DSBs.

Numerous inhibitors targeting the DDR pathways have been integrated into the standard treatment for LA-HNSCC in early-phase clinical trials (Table 16.4) [21]. Currently, these studies mainly focus on mechanisms involved in repairing DSBs. Sometimes, severe toxicities (particularly neutropenia) were observed, when combined with chemoradiation [25, 26]. Combinations with cetuximab and intensity-modulated radiation therapy (IMRT) seemed to be better tolerated [27].

As of now (spring 2024), there are three ongoing phase 1 trials: one assessing adavosertib, a WEE1 inhibitor, in combination with cisplatin chemotherapy preoperatively (group A), as a window of opportunity trial, and in combination with postoperative cisplatin-based chemoradiation (group B) (NCT03028766), another investigating olaparib in combination with definitive accelerated fractionation radiotherapy in patients with stage II-III laryngeal cancer and HPV-negative oropharyngeal squamous cell carcinoma (NCT02229656), and a third trial evaluating

**Table 16.4** DNA damage response (DDR) pathway inhibitors in locally advanced head and neck squamous cell carcinoma (non-exhaustive list)

| Trial (Reference) | Phase | N | Agent | Study description | Activity |
|---|---|---|---|---|---|
| NCT02567422 [25] | I | 43 | Berzosertib (ATR inhibitor) | In combination with chemoradiation | ORR: 72% |
| NCT02585973 [26] | I | 12 | Adavosertib (WEE1 inhibitor) | In combination with chemoradiation | 1-year PFS 90%<br>1-year OS 90% |
| NCT03028766 [28] | I | 9 | Adavosertib (WEE1 inhibitor) | In combination with IMRT + cisplatin weekly in adjuvant | *Ongoing* |
| NCT02555644 [27] | I | 25 | Prexasertib (CHK1 inhibitor) | In combination with chemoradiation or radiotherapy + cetuximab | ORR 80%<br>1-year PFS 83% |
| NCT02308072 [56] | I | 14 | Olaparib (PARP inhibitor) | In combination with chemoradiation<br>**Too toxic** | ORR = 84%<br>1-year PFS 86%<br>2-year PFS 78% |
| NCT02229656 [29] | I | 12 | Olaparib (PARP inhibitor) | In combination with accelerated fractionation radiation | *Ongoing* |
| NCT04533750 [30] | I | 42 | Peposertib (DNA-PK) | In combination with radiation for cisplatin unfit patients | *Ongoing* |

*IMRT* Intensity-modulated radiation therapy, *PFS* Progression-free survival, *OS* Overall survival, *ORR* Objective Response Rate

the use of peposertib, a selective small molecule DNA-PK inhibitor, with IMRT in LA-HNSCC patients who are not candidates for cisplatin (NCT04533750) [28–30].

## *Other Targeted Therapies*

Several targeted therapies are under investigation or have already been validated to improve the efficacy of radiotherapy by acting as radiosensitizers. It is the case of the inhibitors of the epidermal growth factor receptor (EGFR), which is often overexpressed in HNSCC and is correlated with radio resistance and poor prognosis [31]. Cetuximab is a recombinant human/mouse chimeric antibody that binds to the EGFR. As demonstrated many years ago, patients with locoregionally advanced human papilloma virus (HPV)-positive oropharyngeal cancers experience better survival outcomes and tend to be younger [32]. Therefore, it is logical to explore strategies to reduce the intensity of treatments in this patient population to decrease the acute and long-term toxicities. Several de-escalation studies (Table 16.5) have been reported in the last 5 years, comparing high-dose or low-dose cisplatin concurrent with radiotherapy to cetuximab in combination with radiotherapy. The rationale for this combination came from the Bonner trial [33] showing that cetuximab improves survival when combined with radiotherapy with relatively little additional toxicities. However, the three studies, De-ESCALaTE [34], RTOG 1016 [35], and TROG 12.01 [36], all demonstrated similar results, i.e., showing a superior outcome of cisplatin/RT compared to cetuximab/RT, both in terms of locoregional control and survival.

Other targeted therapies are under investigation (Table 16.6), including inhibitors of the PI3K/AKT pathway. The activation of this pathway is linked to three major radioresistance mechanisms in head and neck cancer: intrinsic radioresistance, tumor cell proliferation, and hypoxia [37]. Inhibition of the CDK4/6 pathway

**Table 16.5** De-escalation trials in HPV-positive oropharyngeal cancer

| Trial (Authors) | Control arm | Population | OS (Chemotherapy vs cetuximab) |
|---|---|---|---|
| De-ESCALaTE (Mehanna et al.) [34] | Standard fractionation (70 Gy in 7 weeks) Cisplatin 100 mg/m$^2$ Day 1, 22, 43 | T1N1–T4N3 M0 T3–T4 N0 M0 Non-Smokers or <10 pack-year | 2-year OS rate: 97 vs 89% |
| RTOG 1016 (Gillison et al.) [35] | Accelerated fractionation (70 Gy in 6 weeks) Cisplatin 100 mg/m$^2$ Day 1, 22 | T1–T2, N2a–N3 M0 T3–T4, N0–N3 M0 Smokers included | 5-year OS rate: 85 vs 78% |
| TROG 12.01 (Rischin et al.) [36] | Standard fractionation (70 Gy in 7 weeks) Cisplatin 40 mg/m$^2$ weekly | T1–T2 N2 T3 N0–N2 Exclude N2b >10 pack-year | 3-year FFS rate 93 vs 80% |

*Gy* Gray, *OS* Overall survival, *FFS* Failure-free survival

**Table 16.6** Other targeted therapies in locally advanced head and neck squamous cell carcinoma (non-exhaustive list)

| Trial (Reference) | Phase | N | Agent | Study description | Activity |
|---|---|---|---|---|---|
| NCT03024489 [39] | I | 27 | Palbociclib (CDK4 inhibitor) | In combination with radiotherapy and cetuximab | ORR 84% |
| NCT02537223 [57] | I | 9 | Alpelisib (Class I α-specific PI3K inhibitor) | In combination with chemoradiation or radiotherapy + cetuximab | ORR 67%<br>3-year PFS 78%<br>1-year OS 88.9%<br>3-year OS 78% |
| NCT02113878 [58] | I | 23 | Buparlisib (Pan-PI3K inhibitor) | In combination with chemoradiation | *Ongoing* |

*PFS* Progression-free survival, *OS* Overall survival, *ORR* Objective Response Rate

has also been investigated as a radiosensitizer [38]. A phase I study of palbociclib, cetuximab and IMRT in p16/HPV-negative locally advanced HNSCC patients reported an objective response rate (ORR) of 84% and only 2 of 27 patients had a dose-limiting toxicity [39]. To date, no further clinical development of a CDK4/6 inhibitor concurrently with radiotherapy in LA-HNSCC is ongoing.

## *NBTXR3*

Radio-enhancer nanoparticles represent a category of nanomaterials made to boost the efficacy of radiotherapy [40]. Typically made of high atomic number (Z) elements like gold, gadolinium, or hafnium, they efficiently absorb ionizing radiation and produce secondary electrons or reactive oxygen species upon interaction with radiation. They are inert in the absence of ionizing radiation and only activated by ionizing radiation. NBTXR3 is a novel agent, comprising functionalized hafnium oxide ($HfO_2$) nanoparticles [21]. It is administered intratumorally and remains in the tumor. Efficacy and safety were demonstrated in a randomized trial in soft tissue sarcoma [41]. A phase I study assessed the safety of NBTXR3 in elderly or frail patients with LA HNSCC, ineligible for cisplatin [42]. In the expansion cohort of 56 patients, this study demonstrated the feasibility of NBTXR3 injection, with a manageable toxicity profile: patients received at least 90% of the planned injected volume of NBTXR3 in the oral cavity or oropharynx tumors and IMRT was completed in 50 patients (91%). Prolonged PFS (11.4 months, 6.7-NR months) and OS (18.1 months, 8.9–32.4 months) were observed compared with historical data (PFS ~9 months; OS ~12 months). NANORAY-312 is an ongoing phase 3 randomized study assessing NBTXR3 plus RT versus RT in combination with cetuximab in treatment-naïve, platinum-ineligible elderly patients with LA-HNSCC. Its design is allowing the injection of involved lymph node(s), that might result in higher PFS than observed in the Phase I study.

**Table 16.7** Ongoing Phase 3 trials of immunotherapy in locally advanced head and neck squamous cell carcinoma (non-exhaustive list)

| Trial | Setting | Treatment | N |
|---|---|---|---|
| KEYNOTE 689 [11] | Surgery followed by (chemo) radiation ± pembrolizumab | Pembrolizumab, neoadjuvant, concomitant to radiation and adjuvant | 600 |
| GORTEC [NIVOPOSTOP] [59] | Surgery followed by chemoradiation ± nivolumab | Nivolumab concomitant to radiation and adjuvant | 680 |
| ComPARE [60] | Chemoradiation (oropharyngeal cancer) | Durvalumab neoadjuvant and adjuvant | |
| NCT03811015 [61] | p16-positive oropharyngeal cancer (intermediate risk). Chemoradiation ± adjuvant nivolumab | Nivolumab adjuvant only | 744 |

## Conclusions

Immunotherapy has the potential to improve cancer outcome of patients with LA HNSCC. However, the first reported trials have shown discouraging results. Innovative approaches are needed to investigate the best way(s) to integrate ICI with multimodal curative treatment. For instance, strategies such as better patient selection (PD-L1 expressing tumors) or refinement of treatment scheduling need to be investigated. Additionally, exploring biomarkers such as ctDNA for minimum residual disease detection could further improve patient selection strategies. Hopefully, the ongoing investigations (Table 16.7) and future studies will provide guidance on the optimal utilization of these agents in the curative setting.

Besides immunotherapy, other compounds are currently investigated in locally advanced HNSCC. Xevinapant is a first-in-class, potent, oral, small-molecule IAP inhibitor and has shown promising results in phase II study. Phase III trials are ongoing (Table 16.3).

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# Chapter 17
# Is Reducing the Extent of Local Treatments Justified After Successful Neoadjuvant Therapy?

**Andreas Dietz and Jan B. Vermorken**

## Introduction

Before 1980, the initial treatment of patients with locoregionally advanced stage III or IV (M0) was surgery and/or radiation therapy (RT). Systemic therapy as part of combined modality treatment for locoregionally advanced head and neck squamous cell carcinoma (LA-HNSCC) was introduced mid 1970, initially with single agents (methotrexate, bleomycin or cisplatin), thereafter with cisplatin-based combinations. Neoadjuvant chemotherapy (NCT), also frequently indicated as induction chemotherapy (ICT) given before local therapy became popular in the 1980s, among head and neck surgeons, because of the impressive tumor reductions that could be seen and thereby the expectation of a better survival outcome. However, that enthusiasm waned when no gain in survival was observed in randomized trials comparing local treatments with or without ICT, and randomized trials using concurrent chemo radiotherapy (CCRT) were more promising in outcome [1–4]. Moreover, the MACH-NC meta-analysis suggested superiority of the CCRT over the sequential approach in terms of survival benefit [5, 6]. Although this changed practice in most countries for patients with both resectable and nonresectable disease, interest in the ICT approach remained and even revived when taxanes, targeted agents and immune

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checkpoint inhibitors (ICIs) were integrated in the treatment protocols in the 2000s and 2010s, respectively [7, 8].

The gaining focus on new concepts in neoadjuvant treatments in LA-HNSCC has enhanced the old question: "Is reducing the extent of local treatments justified after successful neoadjuvant therapy?" In particular, the use of ICIs in the neoadjuvant setting, alone or in combination with conventional chemotherapy with subsequential surgery in clinical trials stimulates several questions addressing the standard of resection margins after induction and handling of different degrees of surgical aggressiveness depending on the level of response. Furthermore, the specific biologic behavior of tumor shrinkage and definition of the former tumor bed after induction comes back to the foreground of clinical consideration. Despite technical advances in resection applications, the surgeon suffers from limited intraoperative visibility of vital tumor tissue, especially if the growth is diffusely submucosal or has been altered by induction. In this respect, intraoperative frozen sections are essential for surgical safety. To be able to translate the tumor-reducing effect of a successful induction into the overall result of the therapy, many aspects must be considered. This chapter attempts to determine where we stand on these questions.

## Is There a Benefit of Induction Chemotherapy in Advanced HNSCC?

In 2006, David Adelstein asked the question: "does induction chemotherapy have a role in the management of locoregionally advanced squamous cell head and neck cancer?" [9]. ICT has been an intuitively attractive approach in the management of LA-HNSCC for the last 45 years. As mentioned, high response rates to ICT have been observed in previously untreated patients with this disease. Three cycles of well-tested regimens such as the Wayne State University regimen of cisplatin and infusional 5-fluorouracil (PF) induced an overall response rate (ORR) of 86% and a complete response rate (CRR) of 38% when tested in a multi-institutional study within the Radiation Oncology Group (RTOG) in such patients [10]. Although results with PF plus a taxane (TPF) surpassed those of PF and thereby became a new standard ICT regimen, these responses, are only transient, and, unlike in the lymphoproliferative disorders or in testicular cancer, subsequent definitive surgery and/or (chemo)radiation are still required. As will be seen in the next paragraph, ICT has an established role for organ preservation in advanced laryngeal and hypopharyngeal cancer and the TPF regimen has been validated in that setting. However, there remains uncertainty about the benefit of the sequential approach of ICT followed by CCRT for other indications, even though ICT significantly reduces the occurrence of distant metastases.

The most recent update of the MACH-NC meta-analysis showed a survival benefit for the PF regimen with a HR of 0.90 [95% CI: 0.82; 0.99] but failed to show a similar beneficial effect for the TPF regimen. However, the comparator arm in the

TPF studies in great majority included chemoradiation, while for most the PF studies RT alone was used as a comparator. Moreover, when two TPF trials showing an excess of early deaths by not appropriately selecting patients or not using prophylactic antibiotics or G-CSF, were excluded, there was a significant effect on event-free survival (EFS), but still not on OS [11]. In addition, a recent individual patient network meta-analysis of studies in LA-HNSCC also suggested that further intensifying chemoradiotherapy, by using ICT with TPF could further improve outcomes over CCRT alone [12]. So, although individual randomized studies so far have not given a clear answer as to whether ICT is useful for treatment intensification in daily practice, there are indications from meta-analyses that it might be a valuable approach if the appropriate patients at high risk for recurrence are selected. Therefore, further studies in that direction are still warranted. Outside clinical trials, the utility of ICT is restricted to uniquely pragmatic clinical scenarios, such as unavoidable delay in radiation or severe pain from advanced disease or there is impending airway compromise or neurological dysfunction that necessitate rapid initiation of treatment [13]. Toxicity is an issue of ICT, when there is not much experience with the contemporary ICT regimens. Toxic deaths in the original randomized TAX323 and TAX324 trials on TPF were reported in 2.3% of the patients with unresectable disease patients (TAX323) and in <1% in the study in which two third of the patients had potentially resectable disease (TAX 324) [14, 15]. Crucially in the safe use of TPF regimens is that it is being administered by experienced oncologists, familiar with the necessary protocols and supportive care requirements to ensure patient safety and maximize adherence throughout the treatment [16].

## Role of Neoadjuvant Treatment in Larynx Organ Preservation Programs

Larynx preservation (LP) in locally advanced laryngeal and hypopharyngeal head and neck squamous cell carcinoma (LHSCC) is very desirable, although total laryngectomy (TL) represents an effective treatment strategy. Current treatment options to preserve the larynx and its function include upfront platinum-based CCRT and platinum-based ICT followed by (chemo)radiation in responding patients-The "VA" trial [17] was the first that studied this ICT concept by comparing 3 cycles of PF plus subsequent RT in responding patients (non-responders received surgery plus RT) with standard TL plus postoperative RT in 332 patients with T1–T4, N2–3, M0 larynx cancer. LP was achieved in 64% of the patients in the experimental arm and there was no negative effect on OS, giving support to the safety of this procedure. A similar concept was used in the EORTC 24891 trial [18], which was conducted in 202 patients with T2–T4, N0–3 (N2c excluded) M0 hypopharynx cancer. Again, OS was comparable in both arms of the study and LP was achieved in 62% of the patients in the experimental arm.

With the revival of ICT in the first decade of the twenty-first century, it was to expect that the comparison of TPF versus PF would also be studied in the larynx preservation setting. This was executed by the GORTEC (Group Oncologie Radiotherapie Tete Et Cou) in a phase III protocol in 220 patients with locoregionally advanced larynx and hypopharynx cancer. The primary endpoint of that study was LP, defined as having the larynx in place without tumor, tracheostomy or feeding tube. With a median follow-up 105 months, LP was significantly higher with TPF (at 10 years 70.3% versus 46.5%, p = 0.01), as was the case for laryngeal dysfunction-free survival (LDFS: 63.7% versus 37.2%). However, no significant difference was observed in disease-free survival (DFS) or OS, neither at 5 years, nor at 10 years [19, 20]. Remarkably, statistically fewer grade 3–4 late toxicities occurred with the TPF regimen compared with the PF regimen (9.3% versus 17.1%, P = 0.038).

The second-generation LP trials were executed both in the US (RTOG 91-11; in glottic and supraglottic cancer [2, 21]) and in Europe [22, 23], comparing the ICT approach with the concurrent cisplatin/RT approach and with the alternating PF/RT approach, respectively. The alternating approach, although showing slightly better, but not significantly different, outcome (both in terms of efficacy and toxicity) has not been pursued in most clinics, due to organizational difficulties in delivering this alternating regimen in daily practice. RTOG 91-11 is rather unique in that it is the only trial that compared sequential treatment (PF → RT) with cisplatin-based CCRT and a RT alone arm. The rationale for the second group was based on the enhancement of radiation effects on tumor cells by concurrent treatment with cisplatin [2]. The primary endpoint reported in 2003 was preservation of the larynx, which proved to be in favor of the concurrent cisplatin/RT arm (84% vs 72% with PF → RT vs 67% with RT); also, loco regional control (LRC) was significantly better with the CCRT arm. However, OS was not significantly different among the three arms. These findings are only applicable for stage III or IV larynx cancer patients as were entered in this trial (i.e., T1 and high-volume T4 primary tumors were excluded). At the long-term follow-up analysis, reported in 2013, both chemotherapy regimens significantly improved laryngectomy-free survival (LFS, a changed primary endpoint since 2003) compared with RT alone [2]. The authors indicated that "the larynx was found preserved in most patients with any of the three arms; however, nearly twice as many patients will undergo laryngectomy if treated with induction PF or RT alone instead of concurrent cisplatin/RT" [24], still favoring the concurrent cisplatin/RT approach for that. Important is to mention here that the survival curves started to diverge after 4.5 years with a worse outcome of CCRT relative to ICT (HR, 1.25; 95% CI, 0.98–1.61; P = 0.08). Moreover, an exploratory analysis indicated that for death from causes not related to the study cancer, there was a significant disadvantage for the concomitant group compared with the ICT group (52.8% v 69.8%, respectively, at 10 years; P = 0.03) [21].

The potential need for salvage surgery due to tumor persistence after full perprotocol treatment proved to be a major disadvantage of LP. Late salvage total laryngectomy after CCRT or RT causes major complications and is often not feasible [25, 26]. Therefore, early identification of patients unlikely to benefit from LP

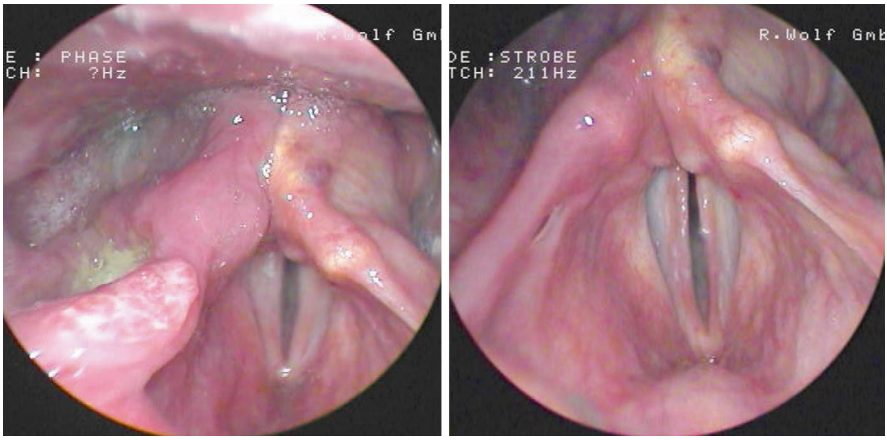


**Fig. 17.1** Patient with T4a hypopharynx carcinoma before (left) and after (right) one cycle TPF, showing complete remission in early endoscopic response evaluation. The patient was treated according to the DeLOS-II protocol (ICT → RT vs cetuximab plus the same ICT → RT in advanced laryngeal/hypopharyngeal cancer resectable only by total laryngectomy; Dietz et al. [28])

attempts is needed to spare the consequences from complete RT or CCRT plus salvage surgery. Since new multimodal treatment protocols and chemotherapies including targeted therapies are emerging [27], further development of LP by induction and radiation remain under consideration. One important field of interest is early response evaluation as a clinical predictor for positive outcome, which was demonstrated in the DeLOS-II-trial by exploring early response by transoral office endoscopy just after the first cycle of induction with TPF/TP ([28]; Fig. 17.1).

According to EHNS-ESMO-ESTRO guidelines, two LP approaches have been validated: concomitant CRT and 3 cycles of ICT followed by RT alone [evidence level I, grade of recommendation A]. As mentioned, the introduction of TPF combinations has proven to be superior to PF schedules and TPF is now considered the standard ICT regimen [I, A]. However, not all patients with locally advanced laryngeal or hypopharyngeal cancer should be offered ICT. Patients with massive larynx cartilage invasion, extra-laryngeal extension or with severely impaired laryngeal function should be excluded from a larynx preservation strategy and offered upfront surgery [III, A] [29].

## Surgical View on New Resection Margins After Neoadjuvant Treatment in HNSCC

The principle of en-bloc resection has been a standard of surgical head and neck oncology. While en-bloc resection remains a key surgical principle, the extension of surgical oncology into more complex anatomical areas and technological advances (i.e., endoscopic mucosal resections, transoral laser microsurgery, TLM, or robotic

surgery, TORS) highlight the need to reconsider the potential merits of piecemeal tumor removal [30, 31]. Piecemeal resections are controversial because they result in fragmentation of the removed specimen, compromising its integrity and complicating confident histopathologic evaluation for the adequacy of excision. While in certain areas of the body, such as the skull base or the larynx, piecemeal tumor removal may be justified by anatomical constraints [32] or functional imperatives [33] (i.e., preservation of voice and deglutition), the apparently increasing use of the piecemeal approach in anatomically simpler and more accessible parts of the human body is more difficult to understand [34]. Quality initiatives by the American Head and Neck Society (AHNS) emphasize the importance of obtaining a negative margin in HNSCC [36]. However, the approach to margin sampling varies considerably from surgeon to surgeon [37, 38]. Trials addressing surgery of oral cavity squamous cell cancer could demonstrate that reliance on margin sampling from the tumor bed was associated with significantly worse local control, most likely owing to narrower margin clearance and greater incidence of positive margins. A resection specimen–based margin assessment is recommended [34, 35].

All mentioned observations are based on experiences with primary surgery in treatment-naive patients. The situation of surgery after NCT is still under strong consideration. The pathological response to NCT can potentially impact the evaluation of the surgical margins, due to a non-centripetal widespread decay of tumor cells throughout the tumor mass that determine a more challenging assessment of tumor infiltration.

Therefore, many surgeons are convinced that any reduction of the original margins after NCT (down staging) must be strictly avoided because of high risk of remnant tumor islands in the former tumor bed. The major surgical opinion is still resection considering the original margins before the application of the NCT (principle demonstration of tumor pattern after NCT are shown in Figs. 17.2 and 17.3).

Furthermore, current literature showed that the historical 5-mm cut-off margins are undue in the definition of “close margin” in TORS, even though there is still no consensus among surgeons regarding the optimal threshold [39, 40]. In this context

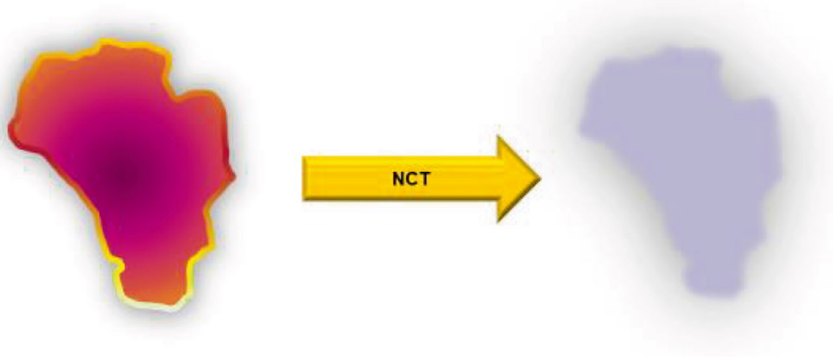


**Fig. 17.2** Principle view on a given HNSCC treated with neoadjuvant chemo therapy (NCT). The ideal result is on the right side: a grey shadow of the vital tumor cell free tumor bed

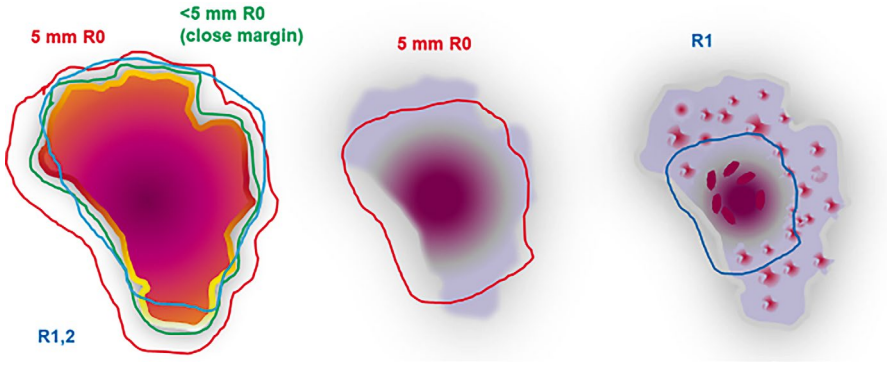


**Fig. 17.3** Tumor pattern for classification of the margins. Picture on the left side: different resection lines to characterize the margins R0 5 mm, R0 < 5 mm, R1,2; picture in the middle: if the tumor shrinks homogeneously without any remnant tumor islands, a closer resection line would end up correctly in R0 (down staging); picture on the right side: tumor shrinkage with remnant tumor islands; a smaller resection line would result in R1 resection (down staging with reduced surgical extent in this situation is not acceptable)

in general in head and neck surgery, there are no studies assessing the role of resection margins after neoadjuvant systemic treatment. Costantino et al. [41] published the first study that assessed the prognostic role of resection margins in patients undergoing TORS after NCT (308 patients; 197 oropharynx, 72 hypopharynx, 39 larynx). The inclusion criteria were as follows: (1) age 18 years or older; (2) diagnosis of squamous cell carcinoma (SCC); (3) completion of at least 2 cycles of NCT; (4) successfully completed TORS. They found that microscopic invasive carcinoma at the inked margin of the specimen is not an independent predictor of tumor control and survival. Moreover, a threshold of 1.25 mm was identified as the most appropriate to define close margins, but no difference was measured after distinguishing negative margins in close and wide margins. Further multicenter prospective studies are mandatory to confirm these results and further data are needed to define if surgical margins have a different role in tumors originating from various anatomical subsites to establish site-specific clinical guidelines for transoral surgery after NCT.

## Neoadjuvant Treatment Before Transoral Surgery of Oropharyngeal Carcinoma

The advent of transoral robotic surgery (TORS) in 2009 has completely changed the surgical approach in the treatment of head and neck cancers [43]. This minimally-invasive technique was first proposed for the management of oropharyngeal carcinoma with encouraging oncological and functional outcomes [43–46] compared with much more comprehensive open surgical approaches. Since then, TORS was

also applied for resections of tumors arising from the hypopharyngo-laryngeal area, with comparable results to transoral laser microsurgery (TLM) [47–49]. Primary tumor ablation with TORS allows to reduce postoperative morbidity, performing a conservative resection that does not necessarily reduce tumor control [50]. It is widely used for the treatment of early head and neck cancers (T1–2), given its potential to obtain complete tumor excision with limited functional impairment [51–53]. Nonetheless, recent studies demonstrated the feasibility of transoral resection also in cases of selected locally-advanced (T3–4) oropharyngeal and hypopharyngo-laryngeal tumors [54–56].

The Se-Heon Kim group (Yonsei University College of Medicine, Seoul, South Korea; Costantino Dec 2023) recently proposed a remarkable protocol that consisted of the administration of NCT followed by TORS, with clinically validated oncological results (198 patients with oropharyngeal SCC, 69% p16 positive). The South Korean NCT regimen consisted of cisplatin (70 mg/m$^2$) by intravenous infusion on day 1 and S-1 (a combination of gimeracil 5.8 mg/m$^2$, oteracil 19.6 mg/m$^2$, and tegafur 20 mg/m$^2$) via transoral route on days 1–14, both repeated every 21 days (Fig. 17.4). After 2 cycles of NCT, response was evaluated by imaging studies based on Response Evaluation Criteria in Solid Tumors (RECIST 1.1) and by endoscopic findings [42].

NCT could achieve complete or partial responses in a large proportion of patients, allowing adjuvant treatment to be tailored on pathological information obtained from the TORS specimen [42, 55–57]. TORS-resection was done considering the visible new tumor borders after NCT, neglecting the earlier mentioned "dogma" of mandatory resection considering the original tumor margins. They reported 54% R0-resection with margins >5 mm, 24% R0 resections with close margins (<5 mm) and 22% R1 resections (mainly positive deep margins). In spite of the general recommendation to go back for subsequent resection in case of R1 resection, no re-resection was performed in this study. That general recommendation is based on the fact that the prognosis of a patient with an initial R1 or narrow R0 resection is not worse if subsequent resection at the correct site leads to the final stable R0 situation [58–60]. In the Yonsey-protocol, adjuvant treatment was given according to the National Comprehensive Cancer Network-Guidelines in 59.1% (13.1% radiation, 46% chemoradiation). The degree of N2b stages moved from 55% before to 23% after NCT, for T4a stages it moved from 20% before to 5% after NCT. N0 moved

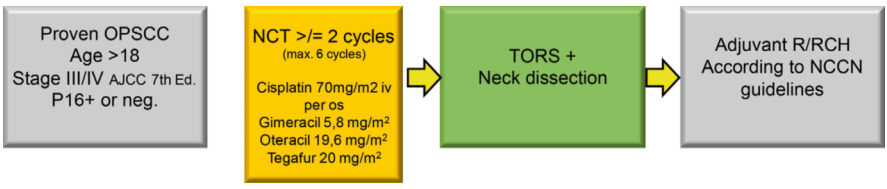


**Fig. 17.4** The "Yonsei" Experience: Neoadjuvant Chemotherapy (NCT) + TORS in oropharyngeal squamous cell carcinoma (OPSCC) (R = adjuvant radiation; RCH = adjuvant chemo radiation) [42]

from 3% before to 45% after NCT. This is remarkable since the authors could show that downstaging of the neck after NCT reduced the need for adjuvant treatment and therefore increased better late functional outcomes.

The median follow-up time was 26.5 months. Estimated DFS rates (95% CI) at 1 and 3 years were 86.6% (81.9–91.7) and 81.4% (75.7–87.6), respectively. Estimated disease specific survival rates (95% CI) at 1 and 3 years were 96.7% (94.1–99.3) and 92.6% (88.4–97.0), respectively. Estimated OS rates (95% CI) at 1 and 3 years were 96.2% (93.4–99.0) and 88.7% (83.4–94.2), respectively. A total of 31 (15.6%) patients showed a disease relapse after a median time of 8 months, but only 12 (6%) patients died of the disease during the study period. A multivariable model was constructed using variables statistically significant at the univariable analysis for DFS. Amazingly, the analysis failed to demonstrate any significance regarding any relationship between positive or close margins and tumor recurrence and death [42].

This monocentric uncontrolled study demonstrates that NCT and TORS can obtain excellent tumor control and survival in locoregionally advanced oropharyngeal cancer and is in support for the new thinking regarding any combination of NCT and surgery in advanced HNSCC. We learned, that NCT seems to be particularly beneficial for locally-advanced tumors (cT3–4) as it improves R0-resectability through a minimally-invasive transoral surgery. In addition, NCT has been offered also to regionally advanced tumors to increase loco-regional control and minimize the requirement for adjuvant therapy. As a matter of fact, typical adverse features that are commonly considered for the indication to adjuvant treatments might be not as relevant in cases of NCT [3, 4]. Surgical resection margins play a dominant role in prognosis of head and neck tumors treated with upfront surgery, including TORS [54]. Indeed, positive margins require re-resection or adjuvant CCRT, while with close margins usually adjuvant RT is recommended [NCCN Guidelines 04.2024] [61]. Sadeghi et al. reported their Montreal-Washington experience [62] in 125 cases with p16-positive oropharyngeal SCCs. The protocol addressed three-weekly cisplatin plus docetaxel followed by transoral surgery. They obtained a complete pathological response (pCR) in 72% of the primary tumors and 54% of the neck disease (overall 44%). Only 5% of patients underwent adjuvant RT or CCRT. The 2-year survival rate was 95%, and the mean swallowing scoring (MDADI) was very high (96.1), compared with MDADI of 78 in the currently most relevant TORS addressing controlled ECOG-ACRIN trial (Transoral Surgery and Low-Dose Intensity Modulated Radiation Therapy in Resectable p16+ Locally Advanced Oropharynx Cancer; E3311 [63]). The Sadeghi late functional outcome data are outstanding. Therefore, the Head and Neck Cancer International Group (HNCIG) proposed recently the controlled NeCTAR trial (Neoadjuvant Chemotherapy before Transoral surgery and Adjuvant Radiotherapy and chemotherapy) for resectable p16 positive oropharyngeal SCCs. The standard arm will be the same protocol without NCT. The proposed NCT includes 3 × cisplatin 75 mg/m$^2$ + docetaxel 75 mg/m$^2$ (contact person for this trial is Prof. Hisham Mehanna, Birmingham, UK). These studies make us very optimistic that a window is opening towards a rationale for reducing local treatment after successful NCT.

## New Options of Neoadjuvant Treatment Including Immune Checkpoint-Inhibitors

Currently, we have learned from several ongoing and finalized NCT trials with integration of immune checkpoint inhibitors (ICIs) about the high potential of this new therapeutic option. Uppaluri et al. presented first preliminary data of two cycles of neoadjuvant pembrolizumab monotherapy before surgery in 36 patients and concluded: Among patients with locally advanced, HPV-unrelated HNSCC, pembrolizumab was safe, and showed pathologic responses in 44% of patients with 0% pCRs. The 1-year relapse rate in patients with high-risk pathology was lower than historically reported. Surgery was technically feasible and no intraoperative and wound healing problems were observed despite the assumed higher tissue blood perfusion due to pro-inflammatory treatment [64]. Following these preliminary data, MSD initiated the KEYNOTE-689, a Phase 3 study of adjuvant and neoadjuvant pembrolizumab combined with standard of care (SOC) in 600 patients with resectable, locally advanced HNSCC. First data are expected in 2025.

L. Zuur and her team from Amsterdam presented the first data of the IMCISION-trial, a non-randomized phase Ib/IIa trial. Thirty-two HNSCC patients were treated with 2 doses (in weeks 1 and 3) of immune checkpoint blockade using nivolumab (NIVO MONO, n = 6, phase Ib arm A) or nivolumab plus a single dose of ipilimumab (COMBO, n = 26, 6 in phase Ib arm B, and 20 in phase IIa) prior to surgery. Pathological response, defined as the %-change of viable tumor cell percentage from baseline biopsy to on-treatment resection in primary tumor, was evaluable in 17/20 phase IIa patients and 29/32 total trial patients (6/6 NIVO MONO, 23/26 COMBO). They observed a major pathological response (MPR, 90–100% response) in 35% of patients after COMBO immune checkpoint blockade both in the phase IIa (6/17) and in the whole trial (8/23), meeting the phase IIa primary endpoint threshold of 10%. NIVO MONO's MPR rate was 17% (1/6). None of the MPR patients developed recurrent HNSCC during the 24.0 months median postsurgical follow-up. As a side note, this is the only trial showing any advantage for combination therapy of PD-1 + CTLA4-blockade compared to anti-PD-1 monotherapy in head and neck cancer [65].

Another encouraging trial was the German CheckRad-CD8 trial from Hecht et al. [66]. Fifty-six patients received a single cycle of cisplatin 30 mg/m$^2$/day on days 1–3 and docetaxel 75 mg/m$^2$ on day 1 combined with durvalumab 1500 mg and tremelimumab 75 mg both at a fixed dose on day 5. Patients with a pCR in the rebiopsy after induction treatment or with at least 20% increase in the intratumoral CD8+ cell density in the rebiopsy compared with baseline continued treatment with radioimmunotherapy (RIT: concomitant durvalumab/tremelimumab/RT). The objective of this interim analysis was to analyze safety and efficacy of neoadjuvant chemoimmunotherapy (NCIT) treatment before RIT. After induction treatment, 27 patients (48%) had a pCR in the rebiopsy and further 25 patients (45%) had a relevant increase in intratumoral CD8+ cells (median increase by a factor of 3.0). On a multivariate analysis, intratumoral CD8+ cell density predicted pCR independently.

Following this observation, in order to assess whether a single dose of ICI added to a single cycle of a platinum-doublet, updated results of the Hecht study were compared with a retrospective cohort receiving the same chemotherapy without immunotherapy. The endpoint of this analysis was the CRR. A total of 53 patients were treated with ICIT and 104 patients with ICT only. Remarkably, CR rates were 60.3% for ICIT and 40.3% for ICT (p = 0.018) [67].

Other highly stimulating small uncontrolled single institution trials focusing on neoadjuvant immunochemotherapy (NICT) with new ICIs for locally advanced resectable oral SCC came from China. The ILLUMINATE-trial was a prospective trial of NICT with toripalimab (PD-1 inhibitor) and albumin-bound paclitaxel/cisplatin (TTP) and was conducted in 20 patients with clinical stage III and IVA oral cavity SCC. The MPR was 60%, including a 30% pCRs and no interference with subsequent surgery. During the median 23-month follow-up, the DFS was 90%, and the overall survival (OS) 95% [68]. In a phase I trial study, 20 patients with locally advanced resectable oral SCC were treated with three cycles of camrelizumab (anti-PD-1 drug) and apatinib (Vascular Endothelial Growth Factor 2-inhibitor) before surgery. Neoadjuvant treatment was well-tolerated, and the MPR rate was 40% (8/20). All five patients with combined positive score (CPS) ˃10 achieved a MPR. A post-hoc analysis showed 18-month locoregional recurrence and survival rates of 10.5% (95% CI: 0–24.3%) and 95% (95% CI: 85.4–100.0%), respectively [69]. Huang et al. published a phase Ib trial with neoadjuvant toripalimab combined with gemcitabine and cisplatin in 23 patients with resectable locally advanced HNSCC (NeoTGP01) [70]. The ORR reached 45%. Eighteen patients underwent successful surgical resection. The R0 resection rate was 100%. The pathological response rates were 16.7% (pCR), 27.8% (MPR, two of five near-pCR), 16.7% partial pathologic response, and 38.8% no pathologic response. Finally, Zhang et al. presented data of a single-center, single-arm, phase 2 trial [71]. Thirty patients with resectable stage III–IVB HNSCC received chemotherapy [albumin-bound paclitaxel 260 mg/m$^2$ (or docetaxel 75 mg/m$^2$) plus cisplatin 75 mg/m$^2$] and camrelizumab 200 mg on day 1 of each 21-day cycle for three cycles, followed by surgery, and adjuvant radiotherapy. The pCR rate was 37.0%, and the MPR was 74.1% (95% CI, 53.7–88.9%). The median follow-up duration was 16.1 months (range, 8.3–28.5), and the disease free survival rate at 12 months was 95.8% (95% CI, 73.9–99.4%). All data presented here are extremely promising and generate relevant hypotheses for future controlled multicenter phase II and III studies to establish NICT before surgery in advanced HNSCC.

With regard to larynx preservation, the traditional domain of NCT, Ferrarotto et al. presented very interesting data of immuno-chemotherapy with pembrolizumab, cisplatin, and docetaxel as single treatment modality for larynx preservation (ICoLP) in 23 patients at ASCO 2023 [72]. Disease control rate was 100% with 74% (17/23) being objective responses and 52% CR; pCR rate was 77.3% (17/22; 1 pt is on-treatment). Six of 17 (35%) patients with pCR developed recurrence, mostly (4/6) within 4 months of pCR, and were salvaged with laryngectomy.

In Germany, the interdisciplinary working group for head and neck cancer (IAG-KHT) started this year the ELOS-trial, a prospective, randomized, open-label,

controlled, two-armed parallel group, phase II multicenter trial in locally advanced SCC of the larynx or hypopharynx (LHNSCC) with PD-L1-expression (CPS) ≥1 and with curable disease by total laryngectomy [73]. ICT with docetaxel and cisplatin (TP) followed by radiation will be compared with the same approach plus a PD-1 inhibitor. Patients will be selected for larynx preservation by application of 2 additional ICT cycles followed by RT (69.6 Gy), if there is endoscopically estimated tumor surface shrinkage (ETSS) ≥30% after the first ICT cycle (ICT 1). Non-responders (ETSS <30% or progressing disease) will receive total laryngectomy and selective neck dissection followed by postoperative radiation or chemoradiation according to the guidelines and the recommendation of a clinical multidisciplinary tumor board. Patients allotted to the intervention arm will receive 200 mg pembrolizumab in three-weekly cycles for 17 cycles (12 months), starting from day 1. Treatment with pembrolizumab will continue in the experimental arm regardless of ETSS status after ICT-1 in both responders and laryngectomized non-responders, independently from subsequent decision on adjuvant therapy after TL (Fig. 17.5).

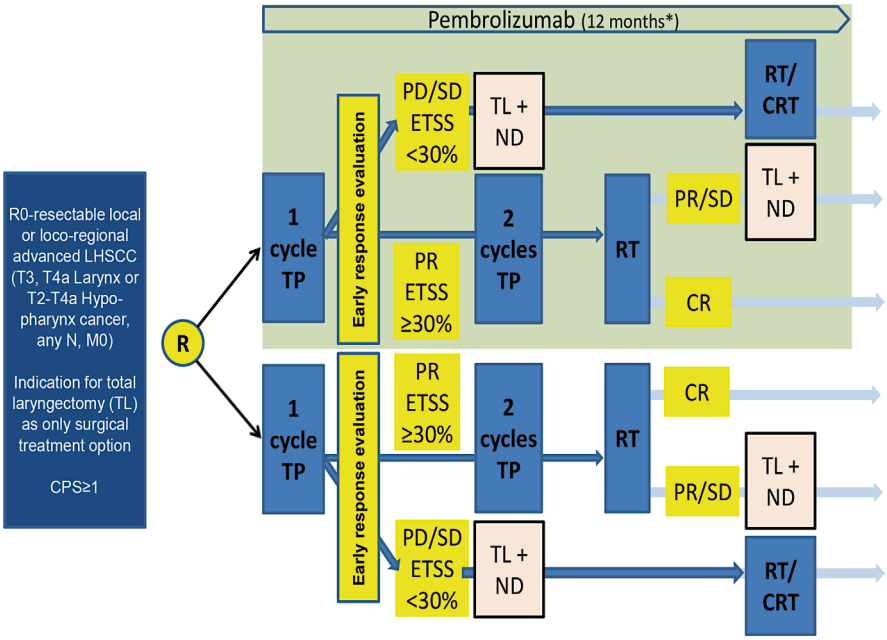


**Fig. 17.5** ELOS-Flowchart—Induction chemotherapy with docetaxel and cisplatin followed by radiation compared to additional PD-1 Inhibition in CPS ≥1 advanced laryngeal/hypopharyngeal cancer suitable for laryngectomy selected after early response evaluation (Andreas Dietz on behalf of the ELOS-working group) [73]. CPS Combined positive score, R Randomization, TP Induction chemotherapy utilizing T: docetaxel, P: cisplatin; Early response evaluation according to DeLOS-II criteria, PR Partial response: 30% endoscopic tumor surface shrinkage (ETSS) after one cycle, PD/SD Progressing disease or insufficient response <30% ETSS, TL Total laryngectomy, ND Neck dissection, RT Radiotherapy, CRT Concomitant cisplatin-based chemo-radiotherapy; endoscopic evaluation. Medication and radiation protocol according to the DeLOS-II larynx organ preservation trial with additional pembrolizumab (over 6 months) in the experimental arm (light green)

The study is based on the encouraging experience of the above-mentioned DeLOS-II-trial [28].

## Current Clinical Research on Intraoperative Margin Assessment

There is no doubt that the bottle neck for future accuracy of surgery after NCT/NICT is still the very limited visibility of tumor margins. The intraoperative assessment of tumor margins of head and neck cancer, especially after NCT or NICT, is crucial for a complete tumor resection and patient outcome. The current standard is to take tumor biopsies during surgery for frozen section analysis by a pathologist after using only hematoxylin-eosin staining. This evaluation is time-consuming, subjective, methodologically limited and subject to a selection bias. Any improvements in staining, intraoperative detection of tumor margins and acceleration of pathologic procedures are therefore of high interest to overcome these limitations. Nevertheless, none of these new methods has any relevance for daily routine assessment yet. Anyway, there are some very interesting scientific developments underway that stimulate surgeons to conduct trials concerning resection margins. The term for this field of research is "intraoperative margin assessment" (IMA).

For example, Pertzborn et al. focused on optical methods such as hyperspectral imaging (HSI). The group aimed to analyze the feasibility and accuracy of an intraoperative HSI assessment on unstained tissue sections taken from seven patients with oral SCC. Afterwards, the tissue sections were subjected to standard histopathological processing and evaluation. Different machine learning models on the HSI data were trained, including a supervised 3D convolutional neural network to perform tumor detection. The results were congruent with the histopathological annotations. Therefore, this approach enabled the delineation of tumor margins with artificial HSI-based histopathological information during surgery with high speed and accuracy on par with traditional intraoperative tumor margin assessment (accuracy: 0.76, specificity: 0.89, sensitivity: 0.48). The authors suggest that HSI in combination with machine learning hyperspectral imaging may be a potential new tool for intraoperative tumor margin assessment [74].

Other groups focus on molecular, Hsp70-specific fluorescence imaging for intraoperative imaging in the resection of head and neck tumors with encouraging ex-vivo data. The following compilation of IMA key technics will give an overview about this growing field of surgical interest (mainly outside of head and neck) [74–81]:

- **Bioimpedance (MarginProb®)** is quick (~5–7 minutes) and a 50% reduction in reoperation rates is achievable despite modest sensitivity and specificity.
- **ClearEdge®** system measures tissue-specific electrical properties (sensitivity ~85%, specificity ~80%).
- Optical spectroscopy techniques such as **hyperspectral imaging (HSI)**

- **Raman spectroscopy**
- **optical coherence tomography (OCT)**
- **spatial frequency domain imaging**
- **fluorescence techniques**
- **confocal microscopy**
- **Hsp70-specific fluorescence imaging**

## Conclusion

In the last few years, NCT and NICT as part of the perioperative transoral surgical treatment concepts of advanced HNSCC is again gaining interest due to increasing (pathological) response rates and promising functional and OS outcomes. NICT showed to be effective in various uncontrolled, small trials and has opened the door to new surgical concepts. However, direct comparisons of NCT vs NICT, including OS data, are lacking. Therefore, the initially raised question "is reducing the extent of local treatments justified after successful neoadjuvant therapy?" can currently not be answered by yes or no. Today, NCT/NICT in combination with transoral surgery is not a standard treatment. However, the topic is highly relevant and should stimulate worldwide the oncologic community to perform NCT/NICT clinical trials focusing on downsizing, better detection of resection margins, the role of R0/R1 resection after neoadjuvant treatment, gaining precision in transoral surgery, limiting the extent of surgical resection, limiting adjuvant treatment and improving the intraoperative surgical view by investigating new technical tools. This seems to be the challenge for improving concepts in head and neck surgery with a focus on better survival and late functional outcomes in our patients over the next decade.

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# Chapter 18
# Non-invasive Biomarkers Predicting Outcome in Patients with Locoregionally Advanced Head and Neck Squamous Cell Carcinoma (HNSCC)

**Marc Oliva, Sandra Llop, Lorena Arribas, and Ricard Mesia**

## Introduction

Locoregionally advanced head and neck squamous cell carcinoma (LA-HNSCC) poses a significant challenge in the field of oncology due to its substantial global morbidity and mortality [1] necessitating a tailored approach for individualized prognosis and treatment.

Tumor tissue sample analysis is the gold standard for the definitive diagnosis of solid malignancies, including HNSCC. The comprehensive pathological examination also encompasses the evaluation of several tissue biomarkers that are not only prognostic but also predictive of response to specific anticancer therapies, and as such, define treatment [2]. However, tissue samples entail several limitations including availability, quantity and/or quality of the tissue required for each marker analysis and the need of invasive procedures (biopsies) with their implications for patients (i.e. discomfort, pain and risk of bleeding or infection). Additionally, relying solely on tumor biopsy biomarkers may not offer a complete representation of the entire

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tumor, limiting our understanding of intratumor heterogeneity and its microenvironment [3].

It is well-known that patients' baseline condition beyond tumor biology and characteristics is also crucial for treatment selection and prognosis. In addition to the tumor tissue itself, local and systemic host-related factors play an important role in determining outcomes and predicting toxicity to anticancer therapies. Patients' inflammatory and nutritional status are known to impact on cancer prognosis, and they can be assessed by evaluating specific biomarkers [4].

In the pursuit of personalized and minimally invasive approaches, the identification of non-invasive biomarkers has emerged as a promising avenue [5]. These biomarkers have the potential to revolutionize prognostic assessments and guide therapeutic decisions without subjecting patients to invasive procedures. Non-invasiveness, defined as avoiding the introduction of instruments into the body, becomes paramount. Blood analysis could be considered non-invasive if obtainable through routine tests during the oncological process.

This review aims to highlight the evolving role of non-invasive biomarkers in LA-HNSCC patients, transcending the traditional reliance on biopsies. We have categorized non-invasive biomarkers into four subgroups: circulating tumor DNA (ctDNA) , inflammatory biomarkers, nutritional biomarkers, and microbiome (Table 18.1). We strive to identify reliable markers that can be readily assessed

**Table 18.1** Summary of non-invasive biomarkers in LA-HNSCC

| Non-invasive biomarker | Source | Prognostic value | Predictive value | Feasibility to use in clinical practice | ESMO evidence level[a] |
|---|---|---|---|---|---|
| ctDNA | | | | | |
| HPV ctDNA [13] | Blood<br>Saliva | Yes | Yes | Yes, but expensive | Level I |
| Tumor ctDNA [19] | | | | Not enough evidence | Level III |
| Inflammatory biomarkers | | | | | |
| NLR [30] | Blood | Yes | No | Cheap and easy to introduce in clinical practice but not enough evidence. | Level IV |
| PLR [35] | | | | | Level IV |
| LMR [36] | | | | | Level IV |
| Nutritional biomarkers | | | | | |
| Sarcopenia [48] | Axial CT L3 | Yes | No | Cheap and easy to introduce in clinical practice but not enough evidence. | Level IV |
| GPS [4] | Blood | | | | Level IV |
| Microbiome | | | | | |
| Microbiome [68] | Stools | No | No | Not enough evidence. Technically challenging for daily clinical practice | Level IV |
| | Saliva | Yes | No | | |

*HPV* human papillomavirus, *ctDNA* circulating tumour DNA, *NLR* neutrophil-to-lymphocyte ratio, *PLR* platelet-to-lymphocyte ratio, *LMR* lymphocyte-to-monocyte ratio, *GPS* Glasgow prognostic score

[a]Level of evidence according to ESMO Clinical Practice Guidelines Standard Operating Procedures CPG Version 2.3; October 2023

through easily accessible biological samples, such as blood, saliva, urine, or stool [6]. This not only holds the promise of enhancing prognostic accuracy but also opens approaches for real-time monitoring and timely therapeutic adjustments.

## Circulating-Tumor DNA: A Promising Biomarker in LA HNSCC

Circulating-tumor DNA (ctDNA) has become one of the most promising non-invasive biomarkers across tumor types due to its potential use in multiple stages of the disease including screening, diagnosis, treatment-response evaluation, minimal residual disease (MRD) detection and disease surveillance [7]. ctDNA is cell-free (cf) DNA released by tumor cells after cell death that can be detected in plasma or other body fluids such as saliva and urine [8, 9], and can be differentiated from other cfDNA sources by its mutational profile, methylation patterns and fragments length [10]. For instance, in HNSCC, both saliva and plasma are feasible for ctDNA detection, with differential detection rates varying based on primary tumor location and disease burden [8].

ctDNA allows the characterization of tumor-specific molecular alterations (i.e. Tumor somatic mutations, copy number alterations and/or chromosomal rearrangements) with the advantage of providing more comprehensive information when compared to tumor tissue, as it is not limited to one specific region or site (primary lesion, lymph nodes or metastasis), therefore accounting for tumor heterogeneity. ctDNA may include the detection of viral sequences/oncogenes in the case of virally-related tumors, such as human papillomavirus (HPV)-associated oropharyngeal squamous carcinomas (OPSCC). A significant proportion of OPSCC are attributable to HPV, especially in Western countries [11, 12]. Tumor-related HPV-derived DNA sequences, and particularly E6 and E7 oncogenes, can be detected in both plasma and saliva using either a polymerase chain reaction (PCR) assay and/or broader techniques such as next-generation sequencing (NGS), and are currently one of the most advanced areas of investigation in HNSCC ctDNA studies [8]. Several studies have demonstrated that HPV) ctDNA is a highly sensitive and specific biomarker for MRD detection and surveillance in HPV-related OPSCC. Chera et al., demonstrated that post-treatment plasma HPV-16 ctDNA detection using a multianalyte digital PCR assay targeting E6/E7 oncogene, has a positive and negative predictive value (PPV/NPV) for recurrence of up to 94% and 100%, respectively in)HPV-related OPSCC treated with definitive chemoradiotherapy [13]. Noteworthy, the lead time between a positive plasma HPV) ctDNA and clinical/radiological evidence can go from weeks to up to a year [13]. Additional techniques such as TTMV (tumor-tissue modified viral DNA), which adds DNA fragmentomic information on top of the oncogene detection, reported a PPV and NPV of 95% for recurrence in patients with HPV positive OPSCC who received radical treatment [14]. Full HPV sequencing using NGS techniques has also been investigated, but despite it is highly specific, it can increase the rate of false positivity compared to

PCR. The use of HPV) ctDNA may not only aid in the evaluation of response but also potentially reduce the costs in patients treated with curative-intent chemoradiotherapy, as its higher PPV and NPV for disease persistence when compared to standard of care protocols (i.e. PET-CT) can lead to a reduction of repeated imaging tests and/or diagnostic/treatment procedures (i.e. biopsies, neck dissections) [15] What is the preferred sample type—plasma, saliva, or both—in this scenario is unclear. In the viral ctHPV substudy of the De-ESCALATE phase 3 trial, a sensitivity of 65% and a specificity of 87% was observed when combining plasma and salivary HPV ctDNA assays in detecting recurrence. These results are lower compared to previous studies conducted by Wang et al. and Ahn et al., where the sensitivity/specificity found when combining plasma and saliva)HPV ctDNA were 86%/100% and 76%/100%, respectively [8, 16].

The investigation of ctDNA in HPV-negative HNSCC is still in an early stage when compared to HPV-positive OPSCC, with very few studies mostly focusing on MRD and surveillance, involving relatively small numbers of patients. The heterogeneous nature of)HPV-negative HNSCC, alongside the predominant presence of inactivating mutations in tumor suppressor genes rather than activating mutations in oncogenes, represents an obstacle to the development of effective biomarkers for this specific subgroup of patients [6, 17]. Two main strategies for ctDNA assessment can be used in these tumors: 1. pre-defined gene panels with known mutations from a prior genomic and/or methylomic analysis of the tumor (tumor-genotype informed analysis) or 2; targeted gene panels that includes the most common mutations in cancers (and/or specifically for HNSCC) or whole exome sequencing applied directly in plasma samples (tumor non-informed assays) [18]. First approach was tested by Flach et al. in a single-center prospective cohort study using the NGS-based personalized tumor-informed assay (RaDaR™) to detect ctDNA in post-surgery samples [19]. Among 17 patients, ctDNA was present in all cases before surgery. Notably, in patients presenting with relapse, ctDNA positivity was consistently detected before clinical progression with lead times ranging from 3.6 to 8.4 months. Mutant ctDNA could be detected at levels as low as a variant allele frequency (VAF) of 0.0006%. Additionally, Kogo et al. also explored the role of tumor-informed ctDNA in monitoring MRD in HNSCC patients who underwent curative treatment. Seven out of 18 patients tested positive and subsequently relapsed [20].

An inherent challenge to the widespread implementation of a tumor-informed approach lies in the substantial cost associated with personalized tumor testing plus the availability of tumor tissue of enough quantity and quality for genomic analysis. As such, tumor-naïve pre-defined panels to be applied to plasma samples are of high interest. The group of Honoré et al. investigated this approach using a pre-defined HNSCC-designed 26-gene panel assay in the setting of MRD detection in LA-HNSCC, showing meaningful specificity and sensitivity in the detection of ctDNA before and after curative-intent treatment. This study included 53 patients and demonstrated an improved 2-year progression-free survival (PFS) in MRD-negative compared to MRD-positive patients: 86.6% (95% CI 73.4% to 100%) compared to 23.5% (95% CI 10% to 55%), respectively. Nevertheless, the

pre-treatment sensitivity using this assay was limited to 72% observed in)HPV-negative tumors [21].

Beyond MRD and surveillance, there is very limited data on ctDNA use for LA-HNSCC, but a few studies have shown its potential role in early diagnosis and as a pharmacodynamic biomarker in the evaluation of response to new drug-combinations in HNSCCs [22, 23].

These findings provide promising insights into how both viral and non-viral ctDNA can serve as a non-invasive biomarker for facilitating screening, diagnosis, surveillance, and potentially personalized treatment decisions. However, further research with larger prospective studies and randomized clinical trials will need to be performed prior to incorporating ctDNA into routine clinical practice.

## Inflammatory Blood Biomarkers: The Role of Peripheral Immune Cells Counts in Predicting Outcome

In the past years, there has been increasing evidence indicating that tumor-related inflammation and systemic inflammation play a crucial role in tumor progression and metastasis [24]. In this context, the evaluation of host factors surrogates of local and systemic inflammatory status has emerged as a potential pathway for identifying new predictive biomarkers that can be easily detected in routine blood, such as is the case of peripheral immune cell count ratios, including neutrophile-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR). However, it is essential to approach the available evidence on this matter with caution, as it primarily stems from retrospective studies.

Neutrophils are known to promote tumor progression, invasion, and metastasis by secreting tumor-promoting growth factors such as epidermal growth factor, vascular endothelial growth factor, interleukin (IL)-6, and IL-8 [25]. Additionally, they can secrete proteases that facilitate tumor cell migration by degrading extracellular matrix and basement membrane proteins [26]. Platelets are also believed to enhance the metastatic cascade not only by triggering the expression of matrix metalloproteinases but also by cloaking metastatic cells, thus shielding them from immune recognition [27]. Conversely, decreased lymphocytes signify a suppression of the immune response, leading to a decrease in antitumor immune cells such as B lymphocytes, NK cells, CD4+ helper T lymphocytes, and CD8+ cytotoxic lymphocytes, as well as a reduction in antitumor cytokines such as interferon and tumor necrosis factor-alpha by tumor-associated macrophages [28]. These mechanisms collectively imply an attenuation of the antitumor specific immune response and may elucidate the relationship between the inflammatory blood biomarkers and prognosis [29].

Several meta-analyses including retrospective HNSCC cohorts have shown a correlation between NLR, PLR and LMR and survival [30–34]. As an example, the meta-analysis from Yu et al. including 5475 patients with non-metastatic disease reported a significant detrimental association between increased NLR and overall

survival (OS) (HR = 1.84; 95% CI 1.53–2.23, p < 0.01)) and disease-free survival (DFS) (HR = 2.18; 95% CI: 1.46–3.24; p < 0.001) [30] The prognostic value of PLR has also been evaluated in a meta-analysis performed by Bardash et al. and they also found an association with worse OS [HR = 1.85, 95%CI 1.35–2.52, p < 0.00001], and DFS (HR = 1.57; 95% CI: 1.25–1.97, p < 0.0001) [35]. Kano et al. found in 285 patients, all treated with chemoradiation with curative intent that a high NLR, a high PLR, and a low LMR were all significantly associated with a decreased overall OS and DFS and showed in multivariate analysis that LMR was an independent prognostic factor [36].

Boscolo-Rizzo et al., tried to evaluate the impact of these ratios independently but also combined: systemic inflammatory marker (SIM) defined as [(neutrophils × monocytes)/lymphocytes]; and systemic immune-inflammation index (SII) defined as [(neutrophils × platelets)/lymphocytes]. Interestingly, the best predictors for OS were SIM (10-year OS = 53.2% for SIM < 1.40 and 40.9% for SIM ≥ 2.46; c-index = 0.569) and LMR (10-year OS = 60.4% for LMR ≥ 3.76 and 40.5% for LMR < 2.92; c-index = 0.568). Moreover, each biomarker was differentially associated with specific patterns of failure. For instance, LMR demonstrated the strongest association with local failure (HR = 2.16; 95% CI: 1.22–3.84), while PLR showed the strongest association with regional (HR = 1.98; 95% CI: 1.24–3.15) and distant failure (HR = 1.67; 95% CI: 1.08–2.58) [37].

Although the meta-analyses provide Level II evidence, it is crucial to acknowledge that they present more weaknesses than strengths. These weaknesses include potential publication biases stemming from retrospective and non-individualized data, significant heterogeneity among included patient cohorts and the absence of consistent cut-off values. Therefore, while inflammatory blood biomarkers are easily applicable in clinical practice due to their low cost and non-invasiveness, the evidence regarding their role in head and neck cancer prognosis is still limited. Standardization in their evaluation, as well as prospective validation in further studies is crucial to confirm their potential role as independent biomarkers.

## Biomarkers Related to Nutritional Status as Predictors of Outcome

Cancer-associated malnutrition is generally acknowledged to differ from deficiency of nutrients in the absence of underlying malignant disease [38]. Depletion in body mass prior to oncological treatment has been shown to have a prognostic significance [39] and it is associated with reduced response and higher toxicity rates during chemoradiotherapy [40–42]. Patients with LA-HNSCC are often at high risk of malnutrition because they present with odynodysphagia; develop acute toxicity during (chemo)radiation; and/or have sequelae after upfront surgery which compromise their oral intake, leading to nutritional deterioration.

High prevalence of inadequate nutritional status and severe inflammatory response have been observed in patients with LA-HNSCC at diagnosis. The levels

of nutrition–inflammation biomarkers (NIBs) can reflect severity of tumor-associated systemic inflammation, identify patients at risk of or established malnutrition and predict patients' outcome. For instance, the Glasgow Prognostic Score (GPS), an inflammation-based model that combines the levels of serum albumin and C- Reactive Protein (CRP), has been associated with OS and RFS in LA-HNSCC patients [43]. Pre-treatment advanced GPS status was correlated with 3-year mortality rates in patients with LA-HNSCC before CCRT [44]. Importantly, this study showed that patients who were already malnourished and with severe inflammation at baseline appeared to have a more aggressive disease and, consequently, worse survival outcomes than those who have adequate nutrition and low inflammation, regardless of tumor stage and performance status.

Recently, accumulating evidence has shown that weight loss occurs mainly at the expense of muscle mass. Sarcopenia—a reduction in muscle mass that can occur regardless of age in patients with chronic diseases, including cancer [45, 46]—has been correlated with disease specific outcomes. A low muscle mass is associated with increased treatment-related toxicity, early treatment failure and higher recurrence-free survival and mortality rates among patients with LA-HNSCC [47–50]. CT analysis of cross-sectional images at the third lumbar (L3) vertebrae is considered the gold standard in the oncological setting to quantify the skeletal muscle mass. Since most of patients with HNSCC do not have a CT-scan performed scan at this anatomical landmark as part of clinical practice, the role of reduced muscle mass in HNSCC came later as compared to other tumor types. In the absence of imaging to the level of L3, some studies used either the third cervical vertebra (C3) [49] or second (T2) [51] or fourth (T4) [52] thoracic vertebra.

Recent publications highlight the importance of implementing measures of strength and functionality in both clinical practice and research as well as the quantification of muscle mass.

Evaluation of malnutrition (using validated tools in oncology population), GPS and sarcopeniashould be routinely performed to identify patients at high risk of poor prognosis and increased toxicity. Evaluation of these biomarkers in prospective studies or in the context of clinical trials are needed and will be crucial to avoid selection bias.

## Role of Microbiome in LA-HNSCC: Current Evidence and Future Perspectives

The human microbiome—the collective genomes and metabolic waste products of all the bacteria, viruses, fungi, protozoa, and archaea that live inside different compartments of the body—has been recently proposed as a new hallmark of cancer given its role not only in tumor development and progression, but also in modulating antitumor immune responses and predicting immune-checkpoint blockade efficacy [53, 54]. Several gut taxa and their metabolites have been linked to response and survival benefit of anti-PD-(L)1 across multiple tumor types [55, 56], while the

use of antibiotics prior to immune-checkpoint inhibitors has been proven detrimental in terms of response rates and survival in cancer patients [57, 58]. As such, there is a growing interest in gut microbiome manipulation—from dietary interventions and probiotics to oral microbial consortia and fecal material transplantation—to boost antitumor immune responses [59].

HNSCC arise from the epithelium and mucosa of the oral and pharyngolaryngeal tract. These compartments are constantly exposed to external aggressions such as smoking, alcohol intake, or infections, which can alter their integrity, causing inflammation and changing the abundance and diversity of oral commensal microbes, also known as dysbiosis [60, 61]. Both Epstein–Barr virus (EBV) and HPV) are well-established etiopathological microbial agents for nasopharyngeal and oropharyngeal carcinomas, respectively, with their distinctive tumor biology and prognostic implications when compared to other HNSCC [12, 62]. Given the preexisting adaptive host immune response against viral-specific antigens, these tumors have a more-favorable immune-contexture, including higher CD8+ tumor infiltrating cells (~30%), a decreased CD4+/CD8+ ratio and higher PD-L1 expression in both tumor and immune cells, which may predispose to a higher chance of response to immunotherapy [63].

But beyond these specific viral–tumor type causality relationships, accumulating evidence suggests a relationship between dysbiosis and HNSCC oncogenesis and progression [61, 64]. Among others, Fusobacterium nucleatum is one of species most increased in oral and tissue samples from patients with oral and oropharyngeal SCC when compared to healthy patients [65], and it has been associated with a higher stage and risk of recurrence in LA-HNSCC, including) HPV-related OPSCC [66, 67]. Preliminary studies have also correlated this taxon with the activation of oncogenic and immune evasion pathways [68]. Regarding the role of both gut and oral microbiome as predictors of response to therapies in HNSCC, data are lacking. For instance, in other tumor types such as esophageal SCC, the dynamics of intratumoral *Fusobacterium nucleatum* seems to be predictive of response to curative-intent cisplatin-based chemoradiation [69, 70]. A prospective observational cohort by Oliva et al. evaluated both oral and gut microbiome in patients with)HPV-related OPSCC treated with curative intent cisplatin-based chemoradiotherapy. While treatment significantly changed the oral microbiome composition and decreased its diversity, it did not seem to have an impact on gut microbiome [67]. Because of the limited number of patients, a correlation with response and/or survival could not be established. However, patients with a higher risk of relapse (stage III) had a higher oral relative abundance of *Fusobacterium nucleatum* and a lower prevalence of the immunotherapy-response-associated species (i.e. *Akkermansia muciniphila*) in their stools [63], indicating the potential relevance of microbiome as an additional prognostic biomarker in this group, who may aid to guide treatment selection (i.e. immune-checkpoint inhibitors-based intensification therapies). In this regard, prospective evaluation of oral and intestinal microbiome was included in the setting of an international chemotherapy-sparing clinical trial evaluating radiotherapy plus durvalumab in)HPV-positive intermediate-risk OPSCC (NCT03410615). However, the study closed prematurely, and the results are pending. The feasibility of gut

microbiome manipulation using oral microbial consortia with in-vitro cultured immunotherapy-response-associated species (MET-4) has also been evaluated in)HPV-related OPSCC by the same group. Preliminary findings revealed that this approach is safe and feasible, and that engraftment of administered bacteria vary by patient but overall was more notable in patients with stage III disease, indicating again a potential opportunity for patient selection for further interventional clinical trial design involving microbiome modulation [71].

## Conclusions

The integration of non-invasive biomarkers into clinical practice for LA-HNSCC patients holds significant promise for enhancing prognostic accuracy, which will aid risk-stratification and potentially treatment selection. Additionally, some biomarkers may be used for real-time monitoring of response and toxicity.

A joint evaluation of different parameters including ctDNA, inflammatory peripheral immune cell count, nutritional indicators and microbiome may provide a more comprehensive understanding of tumor biology, its interaction with the host and the overall response to treatments in terms of outcome and toxicity.

Further research and validation of these biomarkers in larger cohorts and clinical trials, as well as cost-effectiveness assessments, are essential to establish their clinical utility and incorporate them into routine care.

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# Chapter 19
# Innovations in Head and Neck Surgery in the Twenty-First Century


**M. Matthijs Fockens, Jan-Jaap Hendrickx, and C. René Leemans**

## Introduction

The field of head and neck cancer surgery is continuously evolving. In this chapter we describe several recent and important innovations in head and neck surgery that have the potential to improve patient survival and quality of life. Some have already proven their validity and success, whereas others are currently being developed and tested.

## Transoral Robotic Surgery (TORS)

Transoral Robotic Surgery (TORS) has emerged as a significant advancement in head and neck oncology since the FDA approval of the Da Vinci Surgical System in 2009. Presently, TORS finds its main application in two domains within head and neck oncology: addressing cases of unknown primary tumors and resecting primary oropharyngeal (tonsil and base of tongue) cancer. Other indications include resection of supraglottic lesions and parapharyngeal tumors, thyroid surgery and robotic assisted neck dissection.

There has been a notable evolution in managing head and neck squamous cell carcinoma (HNSCC) of unknown primary site (CUP). The incidence of CUP patients in HNSCC has historically been estimated around 2% (depending on work-up and imaging), but with the growing prevalence of human papilloma virus (HPV) infections this figure is increasing [1–3]. HPV-driven oropharyngeal cancers typically have large neck nodes and small or occult primary tumors. The diagnostic

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process for CUP commonly involves ultrasound-guided fine needle aspiration (FNA) and additional cross-sectional imaging, such as contrast-enhanced CT or MRI, often complemented by positron emission tomography-CT (PET-CT) [4, 5]. Furthermore, image-guided surgery (particularly narrow band imaging) has demonstrated efficacy in detecting occult primary tumor localizations, with up to a 35% increase in detection rates following negative PET-CT and MRI scans [6]. Besides these imaging developments, there is mounting evidence supporting the utility of tongue base mucosectomy (TBM) in further enhancing the identification rate of primary tumors (see Fig. 19.1), as indicated by several meta-analyses reporting identification rates ranging from 45% to 64% [7–10].

The additional value of TBM is reflected by its incorporation in several guidelines (e.g. NCCN) [11]. The recent FIND trial, a non-randomized controlled trial for radiotherapy volume deintensification in 22 patients, demonstrated that pharyngeal sparing and unilateral neck radiotherapy were achieved in 45% and 50%, respectively [12]. The whole cohort experienced a reduction in MD Anderson Dysphagia Inventory (MDADI) composite scores. The reduction in pharyngeal radiotherapy was associated with a smaller decline in MDADI swallowing scores (19.3 vs. 39.7), meaning a lower reduction in subjective swallowing difficulties after treatment. With regards to objective Dynamic Imaging Grade of Swallowing Toxicity (DIGEST) scores, the group with the pharyngeal sparing radiotherapy had no worsening of DIGEST scores whereas the pharyngeal radiotherapy showed 60% worsening of DIGEST scores (0% vs. 60%). This study indicates a long-term beneficial effect on quality of life when using TBM in the work-up for CUP [12]. TBM has underscored the necessity for establishing guidelines for assessing resection specimens, emphasizing the importance of adequate specimen marking for pathologists [13]. The MOSES study, which evaluated the value of step serial sectioning, revealed limited added value, suggesting that assessment by a second pathologist may be

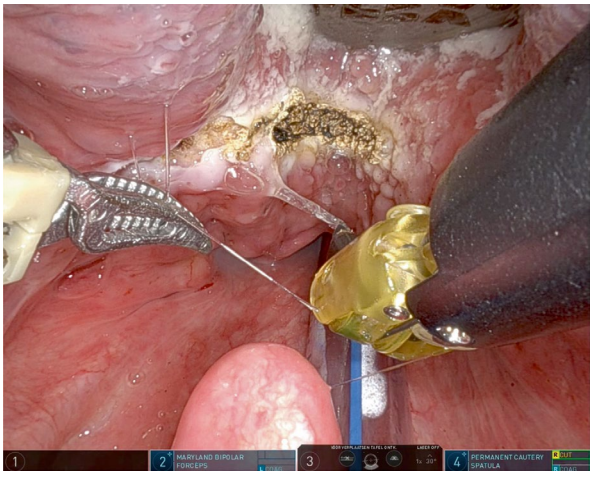


**Fig. 19.1** Intraoperative images of a left-sided tongue base mucosectomy (TBM) with the Da Vinci Xi system. Shown is the first incision with the monopolar cautery. The Maryland Forceps are used to retract the surgical specimen

more beneficial [14]. In our personal experience, the application of p16 immunostaining is very useful to identify small tumors (see Fig. 19.2) [9].

The ORATOR-trial was the first randomized clinical trial that compared surgical treatment (TORS with neck dissection) with non-surgical treatment (radiotherapy +/− chemotherapy) for HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) [15]. Primary analysis after one year showed a significant difference in total mean MDADI score (86.9% for primary RT vs. 80.1% for TORS). However, the difference did not reach clinical meaningful difference. In the surgical group of 34 patients, 10 had only TORS while 16 had TORS followed by radiotherapy and 8 had TORS followed by chemo-radiotherapy. The high number of patients that underwent tri-modality treatment possibly contributed to the worse functional outcome in the surgery group. Furthermore, the subjective swallowing scores for the surgery only group in the ORATOR trial were worse than the same group in the ECOG E3311 trial, which could possibly be explained by the difference in the planned 1 cm margins [16]. Due to an early death caused by an oropharyngeal bleeding, there was a strong recommendation to perform a preventive tracheostomy, which potentially also contributes to worse swallowing.

As the role of TORS within the treatment of HPV-positive OPSCC is increasing, the ECOG-3311 trial was conducted to evaluate its potential to reduce adjuvant treatment [16]. In this trial, patients were assigned to one of four arms based on the pathologic risk factors. Low-risk patients (T1-T2 resected with negative (>3 mm)

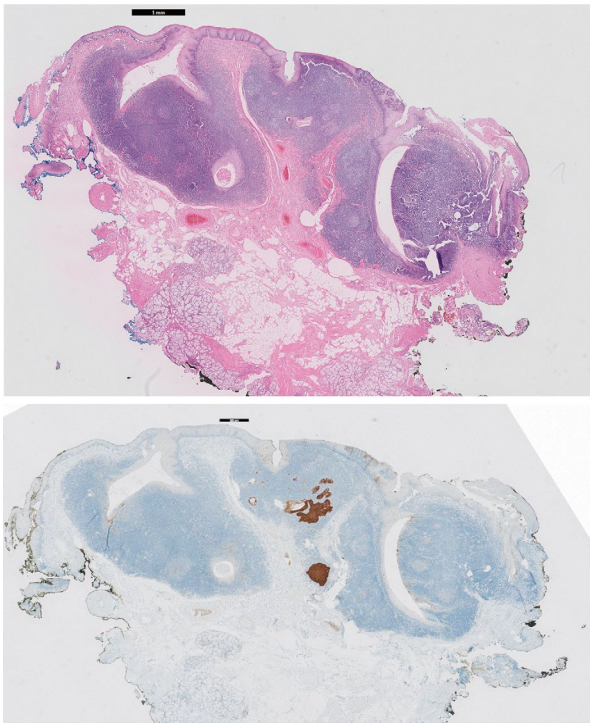

**Fig. 19.2** Microscopic (2 mm) squamous cell carcinoma, at the base of a crypt in the lymphoid tissue at the base of the tongue. (Upper hematoxylin and eosin, lower p16 immunostaining)

margins, N0–1 and no extranodal extension (ENE)) were only followed up (arm A, N = 38). Intermediate-risk patients (T1–2 resected with close (<3 mm) margins, N1-N2 with ≤1 mm ENE or up to 4 positive nodes) were randomized for postoperative radiotherapy (RT) between 50Gy (arm B, N = 100) or 60Gy (arm C, N = 108), stratified by smoking history. High-risk patients (positive margins, >1 mm of ENE, and/or ≥ 5 positive nodes) received concurrent chemoradiotherapy (CRT) (arm D, N = 113). There was no difference in progression free survival (96.9%, 94.9%, 96% and 90.7%, respectively) between the four groups, showing the oncological safety of the de-intensified therapeutic approach. This study opens the path for further studies to de-intensify treatment protocols in HPV-positive OPSCC.

Emerging evidence suggests that patients with HPV-negative tumors treated with TORS with neck dissections +/− adjuvant treatment exhibit more favorable survival outcomes compared to those treated with nonsurgical approaches, with a 5-year overall survival of 71.8% vs. 51.3% [17, 18]. Moreover, a meta-analysis comparing TORS (n = 1302) to intensity-modulated radiotherapy (n = 4322) revealed cumulative survival rates of 91.3% vs. 83.6% and disease-free survival rates of 89.4% vs. 79.6%, respectively [18]. OPSCC of all stages was included, however a greater proportion was advanced (III/IV) (n = 3864) vs. early tumors (I/II) (n = 442). It should be noted that in the TORS group the majority of patients received adjuvant treatment (67.8%), of which half (32.6%) were treated with chemo-radiotherapy. In the radiotherapy group, 81.3% was given concurrent chemotherapy.

The status of margins in TORS remains a topic of ongoing debate. The conventional margins classification (≥5 mm, <5 but >1 mm, ≤1 mm) is presently under evaluation [19]. Various clinical trials employ different cutoff values; PATHOS defines clear margin as >5 mm, while ECOG E3311 and ORATOR2 utilize >3 mm [16, 20, 21]. Additionally, ORATOR, AVOID and a study conducted by the University of Pennsylvania utilize yet other margin specifications (>2 mm) [15, 22, 23]. In the EORTC 1420 on the other hand, clear resection margins are defined as mucosal margins of >3 mm and absence of invasion in the constrictor muscle laterally in tonsillar resections [24].

Recently evidence from the FIND trial suggests the possibility of minimizing or completely avoiding postoperative radiotherapy even in primary tumor settings [12]. In the AVOID trial, a single-arm phase 2 prospective trial, patients (N = 22) with pT1–2 N1–3 HPV positive OPSCC were treated with TORS [22]. For patients exhibiting favorable primary tumor features (e.g. negative margins >2 mm, no perineural invasion, no lymphovascular invasion), the primary site was actively omitted from the radiotherapy planning. In these patients, the primary site received 36.9Gy (standard deviation 10.3Gy). After a median follow-up of 2.4 years (range 8.5–53.8 months), one patient (1.7%) developed a local tumor recurrence resulting in a local control rate of 98.3%. One patient (1.7%) developed regional failure which was salvaged with a neck dissection. Two patients (3.3%) developed distant metastases. In conclusion, the progression-free survival of this study was 92.1% (95% CI 80.2%–97%). These preliminary findings underscore the potential for reducing adjuvant treatments in selected patients, with the ultimate goal of preserving long-term quality of life.

A recent meta-analysis of 5 studies compared swallowing outcomes of TORS (n = 196) and radiotherapy (n = 283) in patients with cT1-2 N0-2 OPSCC [25]. The analysis revealed no significant difference in MDADI-score changes between the two groups. However, it's noteworthy that 54% of surgically treated patient also received adjuvant treatment (RT or CRT), while approximately 80% of radiotherapy patients underwent CRT. The authors concluded that they "did not find a superiority of one treatment over the other".

When interpreting the current literature, there appears to be a subset of patients that can be treated with a single modality treatment that can benefit from the use of TORS as primary mode of treatment, or that gain a survival benefit by the additional treatment modality offered by TORS. [18, 25] Moreover, TORS presents an opportunity to potentially reduce the intensity of treatment for HPV-positive OPSCC patients [16].

These conclusions highlight TORS's beneficial role in the management of OPSCC treatment. However, further studies, both current and prospective, are essential to clarify the most effective treatment approach. These studies should focus on enhancing long-term survival rates while also minimizing the impact on patients' long-term quality of life.

## Sentinel Lymph Node Biopsy

Lymph node status is one of the most important prognostic factors in head and neck cancer. Examination of lymph nodes is generally performed with palpation of the neck and conventional radiological imaging like MRI, CT, ultrasonography, ultrasound-guided fine-needle aspiration cytology, and/or PET-CT [26]. Patients with a clinically negative neck (cN0) have a risk of occult lymph node metastases, as microscopic lymph node metastases remain unnoticed with these imaging modalities. Novel imaging modalities are under development to improve pre-treatment staging accuracy, like MRI with ultrasmall particles of superparamagnetic iron oxide [27]. Although the first results are promising (sensitivity 82–100%, and specificity 77–100%), larger studies and widespread availability are required to implement this technique in the clinic.

In early-stage (T1-T2) oral squamous cell carcinoma (OSCC) treated with transoral surgery, management of a clinically negative neck remains a dilemma [28]. As the risk of occult lymph node metastasis in OSCC is approximately 20–30%, a "watchful waiting" policy may lead to undertreatment and be associated with decreased disease control. Traditionally, an elective neck dissection (END) of the levels most suspectable for lymphatic spread was advised for regional control. However, END does not contain positive lymph nodes in the majority of patients and is associated with morbidity like neuropraxia of the accessory nerve. In recent years, sentinel lymph node biopsy (SLNB) has been adopted as a diagnostic strategy to accurately stage lymph node status.

SLNB evaluates the tumor-specific lymphatic drainage pattern and is aimed at surgical removal of the lymph node(s) that are at greatest risk for occult metastases. The procedure starts with preoperative peritumoral injections of a $^{99m}$Tc-nanocolloid radiotracer, followed by a lymphoscintigraphy and/or single photon emission computed tomography/CT (SPECT-CT) scan to visualize the sentinel lymph nodes (Fig. 19.3). Subsequently, one or more sentinel lymph nodes are intraoperatively identified with a gamma probe and excised for histopathological examination. SLNB is performed in the same surgery as primary tumor resection. Histopathological examination of the sentinel lymph node(s) includes step-serial sectioning and immunohistochemical staining to carefully assess for macrometastases (tumor clusters >2 mm), micrometastases (tumor clusters between 0.2 mm and 2 mm) and isolated tumor cells (tumor clusters <0.2 mm, or single cells). The degree of nodal metastasis (macro-, micrometastases and isolated tumor cells) found with extensive histopathological assessment of SLNs has been correlated with overall survival [29]. In case of tumor positive SLN, subsequent neck treatment is indicated for potential non-SLN lymph node metastases (even in case of isolated tumor cells). Subsequent neck dissection found non-SLN lymph node metastases in 13% for isolated tumor cells, 20% for micrometastasis and 40% for macrometastasis [30]. Neck dissection is considered the treatment of choice for subsequent neck treatment, but radiotherapy can be considered a suitable alternative if adjuvant radiotherapy of the primary tumor site is indicated.

Multiple systematic reviews have evaluated the diagnostic value and accuracy of SLNB in OSCC. Upon comparison with routine histopathology of neck dissection specimen (performed irrespective of SLNB result), the sensitivity and negative predictive value of SLNB were 94% and 96%, respectively [31]. However, follow-up

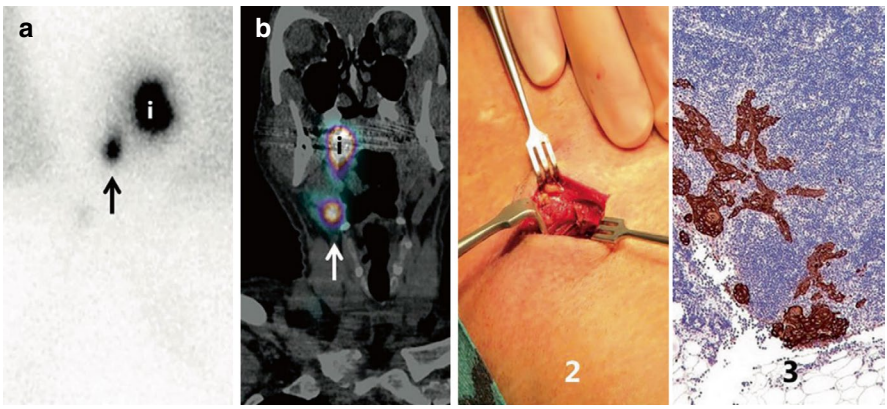


**Fig. 19.3** Steps of the sentinel lymph node biopsy procedure. After preoperative injections (i) with $^{99m}$Tc-nanocolloid around the primary tumor of the tongue, lymphoscintigraphy (1a) and single photon emission computed tomography (1b) are performed to visualize the sentinel lymph node (arrows). Subsequently, the sentinel lymph node is excised through a small incision [2]. Histopathological examination identifies a lymph node metastasis [3]. (Reproduced with permission; courtesy of prof. dr. R. de Bree, UMC Utrecht, The Netherlands)

can be considered a better reference than histopathology of END, as the latter does not include step-serial sectioning to identify micrometastases. Upon comparison with follow-up of the SLNB-negative neck, SLNB showed a sensitivity and negative predictive value of 87–92% and 94–96%, respectively [32, 33]. Two multicenter randomized controlled trials showed that SLNB is noninferior to END in terms of regional recurrence (90.7% vs. 89.6% at 2 years follow-up) and disease-free survival (78.7% vs. 81.3% at 3 years follow-up) [34, 35]. Furthermore, 3 and 5 year overall survival rates were comparable between SLNB and END (87.9% vs. 86.6%, and 82.2% vs. 81.8%, respectively). Interestingly, both trials reported significantly better neck functionality in the SLNB group in the first year. However, multiple studies demonstrated that long-term (>one year) neck and shoulder morbidity, psychological distress, and health-related quality of life appear to be similar for SLNB and END. [36–38] These studies illustrate that long-term neck functionality remains comparable between SLNB and END, rendering the short-term differences clinically insignificant. Adverse events are observed more regularly in patients with OSCC undergoing END when compared to SLNB [39]. In terms of costs, multiple reports have demonstrated that SLNB is a cost-effective strategy when compared to END [40–42].

For OSCC of the floor of mouth (FOM), SLNB has significantly worse sensitivity when compared with elective neck dissection (63% vs. 86%, respectively) [43]. This is attributed to the "shine through" phenomenon, which arises when the hotspot of the primary tumor injections overshine the closely situated sentinel lymph node(s) in level I. Development of new SLNB techniques like MR lymphography (with gadolinium or superparamagnetic iron oxide), CT lymphography, PET lymphoscintigraphy, and contrast-enhanced lymphosonography have tried to tackle this problem, but diagnostic accuracy appears to be inferior to the current standard technique (conventional lymphoscintigraphy and SPECT-CT) [44]. Replacement of $^{99m}$Tc-nanocolloid for a $^{99m}$Tc-tilmanocept radiotracer has shown faster injection site clearance but also lower SLN uptake, and does not prevent the "shine through" phenomenon [43, 45]. Technical improvements are therefore warranted to implement a new imaging modality in standard of care. Until the accuracy of SLNB for FOM tumors has improved, super-selective neck dissection of the preglandular fat pad in level Ib is recommended [46]. This fat pad, located in the triangle of the submandibular gland, the mandibular edge and the anterior belly of the digastric muscle, is the direct drainage site for FOM tumors. This approach has shown a false negative rate of 8.3% and a negative predictive value 96.4%.

An advantage of SLNB is that it may show unexpected drainage to the contralateral neck that otherwise would have been left untreated. In lateralized OSCC, identification of contralateral only and bilateral SLNs was found in 2.0–4.1% and 8.7–20.4%, respectively. In these groups, the contralateral SLN was tumor positive in 2.5% and 6.5% [29, 47, 48]. In a large retrospective cohort study comparing END with SLNB, contralateral regional recurrence was seen significantly more often in the END group (3.8 vs. 1.3%) [49]. Furthermore, the five-year disease-specific survival of patients with positive contralateral nodal metastasis (identified with SLNB or bilateral END) was better than that of patients with contralateral regional

recurrence (88% vs. 42%). Another benefit of SLNB is the potential to stage the previously treated neck, as earlier surgery or radiotherapy may have led to an aberrant lymphatic drainage pattern. The technique has proved to be feasible in OSCC with a treated neck, identifying unexpected drainage in 30–67% patients [50, 51].

Besides improvement of the current SLNB technique (especially for FOM tumors), future research is focused on implementing SLNB procedures in other head and neck tumor sites. A feasibility study demonstrated that flexible endoscopy-guided tracer injection in laryngeal and pharyngeal tumors was successful in the majority of patients [52]. Assessment of lymph node status with SLNB in patients with oropharyngeal, laryngeal and hypopharyngeal carcinomas may lead to a patient-tailored irradiation plan for the neck, in which elective neck irradiation may be given unilaterally or omitted completely [53, 54].

## Image-Guided Surgery

In head and neck cancer surgery, inadequate resection margins are associated with decreased disease control and patient survival [55]. In OSCC, resection margins after conventional surgical excision are positive (<1 mm) in up to 43% and close (1–5 mm) in up to 45% [55]. The location of the close or positive resection margin is in the deep resection plane in up to 87% in some series [56]. Accurate visualization of tumor margins contributes to improved oncological outcomes.

Traditionally, frozen sections of the wound bed after resection have been advocated to intraoperatively assess resection margins, but this method is hampered by sample bias, shows low percentage of clear re-resection margins (57.9%) and does hardly improve disease control or survival [57]. Specimen-driven assessment of resection margins appears to be superior to defect-driven assessment, but relocation of the inadequate margins in the wound bed may be challenging. Paired tagging on both sides of the resection line facilitates relocation and has shown to improve inadequate resection margins in up to 90% of patients, but is also considered time consuming and interfering with surgical workflow [58]. Therefore, extensive efforts have been made to improve resection margins with image-guided surgery.

Image-guided surgery concerns all techniques that improve tumor resection by adequate visualization of tumor tissue and surrounding healthy tissue. Ideally, image-guided surgery informs the surgeon on resection margins of the tumor proactively during surgery (*in vivo*). Imaging of the wound bed (*in vivo*) or the resected specimen (*ex vivo*) can determine the need for additional resections.

Optical coherence tomography (OCT) produces two-dimensional cross-sectional images by detecting back-reflected light. The technique provides real-time information but requires tissue contact. Although OCT is able to distinguish benign and (pre)malignant oral lesions with high accuracy, the technique is not implemented in intraoperative practice as it has very limited penetration depth [59].

Narrow band imaging (NBI) uses blue and green light to enhance visibility of mucosal and submucosal capillaries, allowing *in vivo* discrimination between

normal vascular patterns and aberrant patterns associated with tumor angiogenesis. Advantages of NBI are that the technique is widely available, easy to use, and non-invasive. In laryngeal cancer and its precursor lesions, NBI has an established position in clinical practice as it leads to better visualization of tumor borders, detection of additional lesions, and assists in reaching adequate resection margins. [60] The role of NBI in OSCC appears to be limited to early-stage tumors without submucosal extension, as deep margin assessment is not possible [61].

Spectroscopy imaging uses the unique reflection properties of tissue types to differentiate between normal and malignant tissue. Raman spectroscopy (RS) can provide rapid *ex vivo* assessment by placing a disposable fiber-optic needle probe to measure the distance between the resection surface and the tumor. It is important to recognize that the margin assessment is limited to point measurements. A recent study demonstrated that RS is feasible in OSCC, but the technique is still in early development phase and clinical trials are not yet performed [62].

In recent years, much attention has been directed at intraoperative margin assessment with ultrasound. This technique offers both real-time image-guided resection with adequate deep margin visualization (*in vivo*) and examination of the resected specimen (*ex vivo*). In a study that compared 40 patients who underwent ultrasound-guided resection to 96 patients who underwent conventional surgery for squamous cell carcinoma of the tongue, free resection margins were accomplished significantly more frequently with ultrasound-guided resection (55% vs. 16%) [63]. Ultrasound-guided resection also decreased the need for adjuvant radiotherapy (10% vs. 21%), but the rate of re-resection was comparable (10% vs. 9%). Assessment of specimen resection margins with ultrasound shows near similar margins when compared with histopathology (mean absolute difference 1.1 mm) [64]. Ultrasound assessment of tumor thickness and identification of surrounding large caliber vessels has been described in OPSCC to aid TORS. Currently, its application is limited to clinical trials. As the pharynx is a confined space with little room for the ultrasound transducer, development of a smaller transducer is an important hurdle to overcome.

*Ex vivo* margin assessment with magnetic resonance imaging (MRI) for OSCC has been examined in small studies. The technique appears to be feasible, with high contrast between tumor and healthy tissue and acceptable timeframe for margin assessment (within 30 minutes after resection) [65]. As MRI was not sensitive in detection of margins less than 5 mm, its role in intraoperative margin assessment is currently not suitable for clinical workflow. A recent systematic review concluded that ex vivo margin assessment in OSCC with MRI was not superior to ultrasound, which is preferable as it is inexpensive, widespread available, and reproducible across studies [66]. Furthermore, a Danish prospective trial is currently comparing diagnostic 3D ultrasound and MRI for squamous cell carcinoma of the tongue [67].

Fluorescence-guided surgery (FGS) is a promising technique to improve resection margins (Fig. 19.4). Preoperatively, a fluorescent contrast agent that accumulates in tumor tissue is given. To overcome non-specific tracer uptake, untargeted tracers like 5-Amino-Levulinic-Acid (5-ALA) or indocyanine green are being replaced by tumor-specific tracers like cetuximab and panitumumab [68, 69]. These

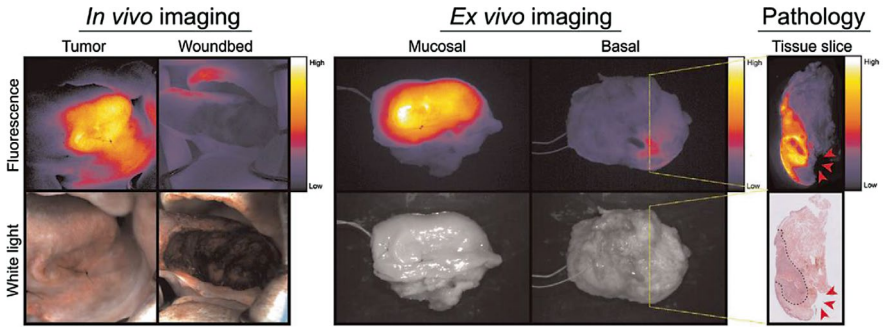


**Fig. 19.4** Fluorescence guided surgery with cetuximab-800CW allows intraoperative *in vivo* imaging of oral squamous cell carcinoma of the tongue. After resection, the wound bed does not show any signs of residual tumor. Back table *ex vivo* specimen imaging identifies a close margin on the deep (basal) border. Postoperative histopathology confirmed this close resection margin. (Reproduced under Creative Commons Attribution License 4.0 (CCBY) from Noorlag, R., Bree, R. de & Witjes, M. J. H. Image-guided surgery in oral cancer: toward improved margin control. Curr. Opin. Oncol. 34, 170–176, 2022)

tumor-specific tracers are coupled with the fluorescent dye 800CW, which allows real-time intraoperative visualization of tumor tissue with near-infrared cameras [70]. In a trial with 65 OSCC patients FGS with cetuximab-800CW enabled *in vivo* imaging for surgical guidance (including identification of previously unknown satellite (pre)malignant lesions) and excellent *ex vivo* identification of positive resection margins (sensitivity 100%, specificity 85.9%, positive predictive value 58.3%,negative predictive value 100%) [71]. Besides a role in tumor resection, FGS can contribute in free flap surgery to evaluate the patency of a vascular anastomosis. Intraoperative indocyanine green angiography (ICGA) has shown to significantly decrease total flap failure rate [72]. Despite its potential benefits, FGS faces several challenges including development of optimal fluorescent contrast agents with minimal side effects, standardization of imaging protocols, and integration into routine clinical practice.

## Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) and 3D Printed Models

Free tissue transfer is the preferred technique for reconstruction of large defects after ablative surgery in the head and neck, especially when large amounts of bone (mandibula and maxilla) need to be removed. In recent years, advances with computer aided design/computer aided manufacturing (CAD/CAM) and three-dimensionally (3D) printed models have greatly improved functional outcomes, while maintaining adequate oncological outcomes in terms of resection margins and recurrence rate [73, 74].

Reconstruction of the mandibula or maxilla is important for dental occlusion, masticatory function, esthetics, and overall quality of life. Osteocutaneous reconstruction typically involves the use of a fibula free flap, scapular tip free flap, or deep circumflex iliac artery free flap. In mandibular and maxillar tumor surgery, the traditional approach relied heavily on a "free-hand" technique: tumor resection, titanium plate alignment, and free flap shaping and orientation were performed at a best estimate, depending heavily on the surgeon's expertise. As a result, the functional outcomes were difficult to predict.

The introduction of 3D techniques in head and neck surgery started with conversion of tissue on computed tomography (CT) scans into 3D anatomic models (Fig. 19.5). The extent of the tumor, its surrounding facial bone(s), and the designated tissue for free flap reconstruction can be segmented into 3D models. Subsequently, 3D printing of these segmentations into solid models provides multiple ways to improve surgical outcome. Fixation of an osteocutaneous free flap for mandibular reconstruction is performed with a titanium reconstructive plate. A printed 3D model of the mandibula allows the surgeon to prepare the reconstructive plate prior to surgery or intraoperatively if sterilized. Advantages include selecting the adequate size and shape, choosing locations for screw placement, and prebending the plate along the curvature of the mandibula. This approach has shown to decrease surgery time, improve strength of reconstructive plates (due to less manipulation), and shorten free flap ischemia time [75, 76]. Furthermore, functional

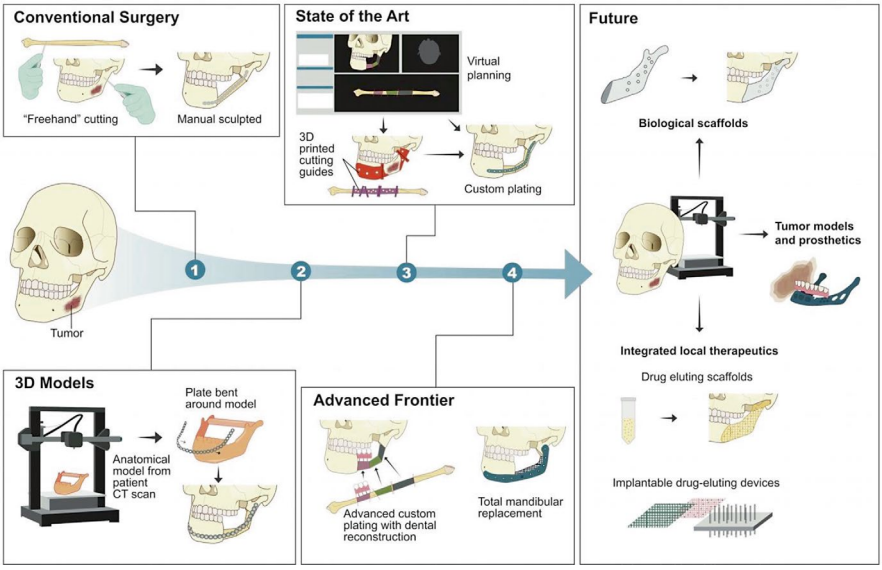


**Fig. 19.5** Evolution of computer aided design and computer assisted manufacturing in head and neck cancer reconstruction. (Reproduced with permission from Nyirjesy, S. C. et al. The role of computer aided design/computer assisted manufacturing (CAD/CAM) and 3- dimensional printing in head and neck oncologic surgery: A review and future directions. Oral Oncol. 132, 105976, 2022)

outcomes are improved as use of pre-bended plates has shown greater bone-to-bone contact on postoperative imaging, indicating improved long term bone consolidation. In case of gross tumor involvement on one side of the mandibula, a 3D segmentation model that mirrors the unaffected side can guide the placement of the reconstructive plate after tumor resection.

Virtual surgical planning is an encompassing term for 3D techniques that enhance surgery. In midfacial tumors, computer assisted surgery has shown to improve resection margins when compared with conventional resection [73]. In mandibular resection, computer-aided design of osteotomies on the ablative segment and the free flap bone help to accomplish optimal reconstructive geometry. This is achieved with 3D printed cutting guides, which are placed on the bone and guide the angle of the osteotomies. Virtual surgical planning has shown clinical benefit with improved osseous union, more accurate osteotomies and fewer osteotomy revisions. It also leads to a significant decrease in operative time and free flap ischemia time [77]. These results directly correspond with a decreased risk of postoperative complications (including free flap failure, surgical site infection, fistula, hemorrhage, partial flap necrosis, and wound dehiscence) and shortened length of hospital stay [76, 78, 79]. Furthermore, functional outcomes like dental occlusion and normal dietary intake are improved with virtual surgical planning when compared to conventional technique [75, 80]. Lastly, there are patient-specific situations in which virtual surgical planning may be beneficial, such as integration of prior radiotherapy planning to perform osteotomies beyond irradiated parts of the bone [81]. Although virtual surgical planning has been proven to improve surgical outcomes, it is important to recognize that the technique is associated with increased costs due to collaboration with commercial parties [82]. These costs are balanced by shorter operation times and may be decreased with development of in-house 3D design workflow with open source software [83]. Design of titanium reconstructive plates can also be incorporated in virtual surgical planning, specifying the number, location, and angle of the screw holes. Custom-made contoured plates eliminate the need for pre-bending (reducing the risk of plate fracture), and have shown to result in fewer revision surgeries or removals of osteosynthesis material [84].

Dental rehabilitation following ablative surgery of the mandible or maxilla plays a crucial role in enhancing functional recovery and quality of life. Osseocutaneous flaps with osseointegrated dental implants are currently considered the best method to reach optimal function. The timing of implant placement remains a subject of controversy, as primary placement carries the potential risk of flap failure, other postoperative complications, or radiotherapy scattering attributed to titanium implants. The use of CAD/CAM techniques emerges as a valuable tool in this context [85, 86].

Future directions for CAD/CAM techniques in head and neck surgery include bioprinting with biomaterial that can incorporate live cells, or the incorporation of drugs and radioactive seeds into 3D printed implants for localized delivery and therapeutic purposes [87].

## Neo-adjuvant Immunotherapy

In recent years, the development and implementation of immunotherapy has rapidly changed the landscape of cancer treatment.

Immune checkpoint inhibitors (ICI) boost the body's antitumor immune response by targeting pathways involving the interaction between tumor cells and T-cells [88]. Tumor cells have developed mechanisms to avoid recognition and destruction by T-cells. Furthermore, antitumor immune responses are restricted by immune exhaustion. This is a process in which persistent antigenic load causes exhausted T-cells to upregulate inhibitory receptors (also known as immune checkpoints) that limit their own effectiveness. Immune checkpoint inhibitors target the receptors and initiate an immune response to tumor cells. Important T cell surface receptors associated with immune inhibition include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Ipilimumab is a T-cell activator that restrains the inhibitory signal of CTLA-4, whereas pembrolizumab and nivolumab target the PD-1 receptor to block binding of PD-L1 and PD-L2 and induce the T-cell antitumor response.

Traditionally, patients with unresectable locoregional recurrence and/or distant metastases (R/M) were offered chemotherapy and/or radiotherapy as palliative treatment, and had a poor prognosis. ICI's have showed OS benefit in platinum-resistant R/M HNSCC (Checkmate 141) and as first-line treatment for R/M HNSCC (Keynote-048). Subsequently, nivolumab and pembrolizumab gained approval for R/M HNSCC in 2016 and in 2019, respectively [16, 89]. Combined blockade of PD-L1 and CTLA-4 with durvalumab and tremelimumab has not shown to improve OS in the R/M population [90, 91].

As ICI showed promising results in R/M HNSCC, multiple trials are investigating its potential in the (neo)adjuvant setting for curative HNSCC treatment [92]. The sequence and timing of ICI is a subject of debate and is being investigated by several studies. Immunotherapy may be beneficial in surgically treated patients in several ways, including reduction of primary tumor size or metastases, conversion of unresectable tumor to resectable tumor, or allowing preservation of organ function.

In a nonrandomized phase Ib/IIa trial comparing neo-adjuvant monotherapy of nivolumab with neo-adjuvant combination of nivolumab and ipilimumab, a major pathological response rate (defined as 90–100% response) was observed in 17% of patients in the monotherapy group and 35% of patients in the combination group [93]. Furthermore, the study demonstrated that FDG-PET prior to surgery adequately identified patients with major pathological response. The authors concluded that neo-adjuvant immunotherapy is safe and does not postpone start of standard treatment.

A recent systematic review of 24 studies concerning 1092 HNSCC patients analyzed the effect of neo-adjuvant PD-1/PD-L1 inhibitors [94]. Objective response rate (ORR; defined as percentage of patients with a complete or partial response according to Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1) guidelines) was reported in 25.8% of patients. In the meta-analysis, the ORR for patients who received immunotherapy or immunochemotherapy was 37%

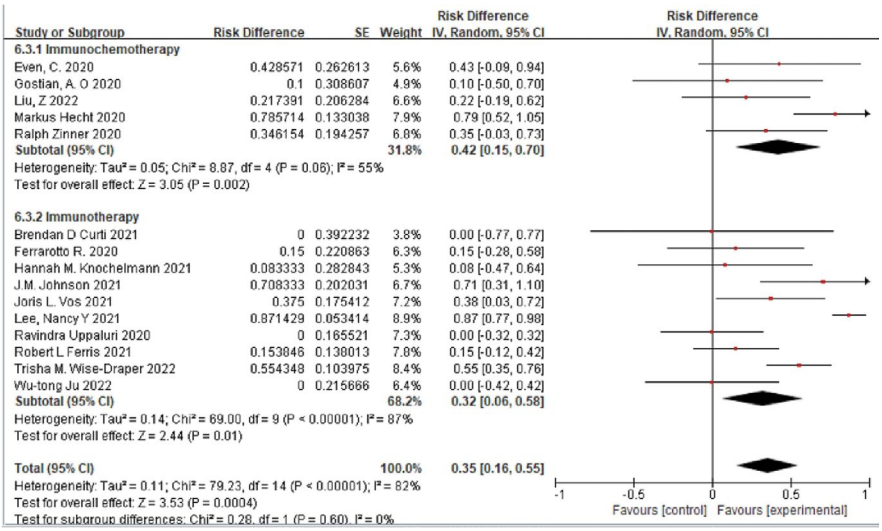


**Fig. 19.6** Forest plot of the objective response rate (ORR; defined as percentage of patients with a complete or partial response according to Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1) guidelines) of neo-adjuvant immunotherapy and immunochemotherapy. The ORR of all patients who received either immunotherapy or immunochemotherapy was 37%. In the subgroup analyses, the ORR was 22% for immunotherapy and 61% for immunochemotherapy. (Reproduced with permission from Chen, S., Yang, Y., Wang, R. & Fang, J. Neoadjuvant PD-1/PD-L1 inhibitors combined with chemotherapy had a higher ORR than mono-immunotherapy in untreated HNSCC: Meta-analysis. Oral Oncol. 145, 106479, 2023)

(Fig. 19.6). Subgroup analysis showed that neoadjuvant immunotherapy combined with chemotherapy has a higher ORR than neoadjuvant immunotherapy alone (ORR 61% vs. 22%). It is important to recognize that the ORR of immunotherapy may be underestimated as imaging studies may be influenced by inflammatory pseudoprogression, a benign process which can mimic tumor growth. One-year survival in 585 evaluable patients was 84% and the 1-year progression-free survival was 82%. Grade 3–4 adverse reactions were observed in 35% of patients, with a slightly non-significant trend towards more adverse reactions in the immunochemotherapy group (42% vs. 35% in the immunotherapy group).

## Conclusion

In summary, innovations in head and neck cancer surgery aim to enhance treatment outcomes in terms of survival and quality of life for patients. We have explicated five key areas (transoral robotic surgery, sentinel lymph node biopsy, image-guided surgery, computer-aided design, and neo-adjuvant immunotherapy) at the forefront of technological advances. Continuous research will offer promising prospects for the future of this field.

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# Part III
# Recurrent/Metastatic (R/M) Disease

# Chapter 20
# 50 Years of Systemic Therapy: What Have We Gained?


**Petr Szturz and Jan B. Vermorken**

## Introduction

Systemic anticancer therapy has become an integral part of palliative approach in patients with head and neck squamous cell carcinoma (HNSCC) and has often been used in the locoregionally advanced setting as part of a multimodality management. The benefits of not only systemic therapy but also other existing modalities become particularly apparent when comparing the outcomes with those of untreated cases. In the year 2000, Kowalski and Carvalho showed that in a population of HNSCC patients treated between 1953 and 1990, overall survival (OS) ranged from one day to 58.3 months with median being 3.82 months. The OS correlated with patients' performance status, and 1-year OS reached 12.9% in those aged less than 70 years with good performance status [Eastern Cooperative Oncology Group (ECOG) of 0–2] [1]. Another measure, which partially reflects advances in systemic therapy along with progress in other treatment approaches and disciplines, including imaging, has been provided by studies analysing survival changes across different periods. Pulte and Brenner demonstrated gradual improvement in 5-year relative survival rates from early 1980s to early 2000s, and this occurred almost irrespectively of tumour site of origine (oral cavity, oro-, and hypopharynx). The greatest leap forward was observed in tonsil cancer, probably relating to the human papillomavirus (HPV) -driven oropharyngeal cancer pandemic. The only two locations where the outcomes remained unchanged were the lip, with high cure rates

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exceeding 90%, and the larynx where approximately two thirds of patients were still alive after 5 years of follow-up after diagnosis [2].

Over the past five decades, it has become evident that our understanding of and approach to HNSCC have evolved. Dichotomization in HPV-positive oropharyngeal carcinomas and other, HPV- negative cases reflects a different disease biology and is further addressed in another chapter of this book (see Chap. 15). However, this is not all. The patient population has changed as well, marked by an increasing number of patients aged 65 years or older, both in absolute and relative terms. While at present, 38% of HNSCC cases are diagnosed in the elderly population per year, this proportion is expected to double in the coming 20 years [3]. Finally, the landscape of anticancer modalities has seen several substantial improvements. The field of surgery has adopted new organ sparing techniques, mini-invasive approaches, the use of lasers, and robotic assistance, all of which improve patient outcomes and reduce recovery times. Similarly, advancements in radiotherapy have enabled more precise targeting, better sparing of normal tissues, and higher effective local doses. The third modality, systemic therapy, will be scrutinized in this chapter, providing insights into traditional cytotoxic chemotherapy, targeted drugs, and modern immunotherapy.

## How Systemic Therapy Has Developed in the Recurrent and/or Metastatic Setting

Throughout the 1970s, 1980s, and 1990s, cytotoxic chemotherapy was the primary treatment available in routine practice for patients with recurrent and/or metastatic (R/M) HNSCC. While the early period was characterised by single-agent regimens, since the 1980s, combination schedules have moved to the forefront of clinical interest. After the turn of the twenty-first century, the first trials began investigating targeted drugs, namely antibodies against the epidermal growth factor receptor (EGFR/ErbB). Their assumed benefit was translated into high-level evidence for extending OS only several years later, following the final analysis of the EXTREME (Erbitux in First-Line Treatment of Recurrent or Metastatic Head and Neck Cancer) phase III trial. It took another decade to introduce an additional, new class of agents, dubbed "immune checkpoint inhibitors" to clinical practice. These agents exert their anticancer potential indirectly by enhancing tumour recognition by the immune system and are now used in both the first and second lines of palliative treatment. Such breakthrough discoveries in the field of immunotherapy have sparked new hopes for long-term survivorship and the development of drugs circumventing resistance of tumour cells to traditional cytotoxic agents. This was partially proved in the second-line CheckMate-141 and Keynote-040 trials and later also in the first-line Keynote-048 trial. Immunotherapy using immune checkpoint inhibitors has been in the spotlight of researchers since more than 10 years and still has to offer numerous trial opportunities exploring various agonists and antagonists acting at different stages of the immune response. These discoveries have been paralleled, as

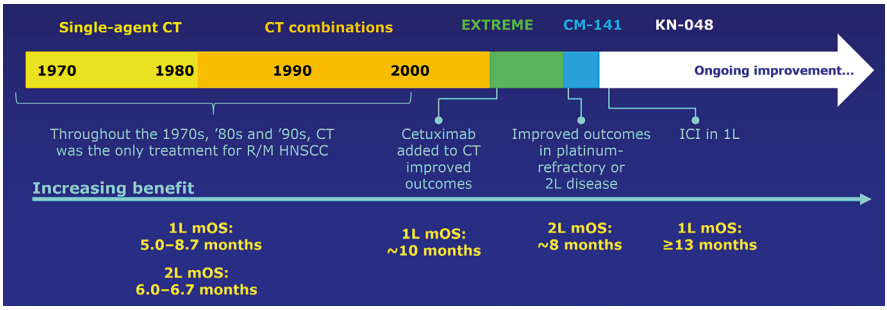


**Fig. 20.1** Development of systemic therapy in recurrent and/or metastatic squamous cell carcinoma of the head and neck (R/M HNSCC) [4–16]. *CT* chemotherapy, *mOS* median overall survival, *ICI* immune checkpoint inhibitor, *CM-141* CheckMate-141, *KN-048* Keynote-048

one would expect, by continuous improvements in median OS which doubled in the first-line to at least 13 months, while some benefits were also observed in the second-line, with survival extending from around 6 to 8 month (Fig. 20.1) [4–16].

## *Cytotoxic Chemotherapy and Targeted Drugs*

Although single-agent methotrexate emerged as a standard of care in early clinical studies, it soon became clear that the highest anticancer potential is associated with cisplatin, particularly in combination regimens [4, 5]. Indeed, relative to single-agent therapies, multiagent schedules yielded superior response rates (platinum/5-fluorouracil vs. platinum: 32% vs. 17%), duration of response (4.2 months vs. 6.7 months, respectively), and OS (5.7 months vs. 6.7 months, respectively), albeit at the price of higher acute toxicity with potential negative impact on quality of life [5–7]. An important step forward was the demonstration of equipotency of 5-fluorouracil and paclitaxel in combination with cisplatin because the paclitaxel-based combination produced significantly less severe acute mucositis (31% vs. 0%) [8]. Unfortunately, the results of the latter trial came too late to influence the design of the landmark EXTREME trial which used 5-fluorouracil and completed enrolment of 442 eligible patients in a record time of one year. Published in 2008, EXTREME established a new standard of care for first-line treatment, comprising cetuximab, platinum, and infusional 5-fluorouracil, which became the standard comparator arm for future trials [9]. This was the reason why the subsequent large Keynote-048 phase III did not adopt a taxane in its chemotherapy backbone [10].

Successful blockade of the EGFR signalling pathway was a breakthrough in the management of HNSCC leading to three approvals by the US Food and Drug Agency including bioradiation with the EGFR inhibitor cetuximab in locally advanced disease, cetuximab monotherapy in the second-line palliative care, and platinum/5-fluorouracil combination with cetuximab in the first-line palliative

systemic treatment [9, 17–20]. In addition, an analogous antibody to cetuximab, nimotuzumab, gained approval for HNSCC in Cuba, India and China, and many other countries outside the US and Western Europe. In the locally advanced setting, cetuximab has not been the only option as an adjunct to radiotherapy but served as an alternative to cisplatin and the carboplatin/5-fluorouracil doublet, albeit its role has diminished over time due to reports of its inferiority with respect to cisplatin, particularly in oropharyngeal carcinoma [21–23], but basically across all primary HNSCC disease sites [24]. On the other hand, the introduction of cetuximab to the first-line palliative setting represented a notable milestone because it was for the first time that a clinically and statistically significant benefit in OS was demonstrated in the R/M setting and later on confirmed in real-world practice [9, 25]. It is fair to say that EXTREME has remained the only successful implementation of an EGFR inhibitor in the first-line R/M setting, as other attempts failed, including a similarly designed phase III trial with cisplatin/cetuximab combination and the recent TPExtreme trial which, despite not meeting the primary endpoint, showed that replacing 5-fluorouracil with docetaxel may still be a reasonable choice in selected patients (Table 20.1) [26, 27].

The EXTREME trial revealed that adding cetuximab to the platinum/5-fluorouracil doublet in the first-line R/M-HNSCC not only leads to improved progression-free survival (PFS) and OS but also nearly doubles the response rate, reduces pain and swallowing problems, has no negative impact on quality of life, and appears to be effective regardless of HPV or p16 status [9, 28, 29]. However, although the response rate doubled, the addition of cetuximab did not lead to an increase in the median duration of response, and the 5-year survival figures stayed remarkably low, with only six patients alive at 5 years out of the 222 that started treatment in the cetuximab arm of the trial [30]. The EXTREME regimen has thus become a new standard of care and has remained so until the present, although for a smaller patient population as will be explained below. Despite all these

**Table 20.1** Randomized trials exploring cetuximab with platinum in the first-line recurrent and/or metastatic squamous cell carcinoma of the head and neck [9, 26, 27]

| Study, First Author and Year of Publication | N | Regimen | ORR (%) | PFS (months) | OS (months) |
|---|---|---|---|---|---|
| ECOG 5397, Burtness 2005 | 117 | Cisplatin + cetuximab | 26[a] | 4.2 | 9.2 |
| | | Cisplatin + placebo | 10 | 2.7 | 8.0 |
| EXTREME, Vermorken 2008 | 442 | Cisplatin/carboplatin +5-fluorouracil + cetuximab | 36[a] | 5.6[a] | 10.1[a] |
| | | Cisplatin/carboplatin +5-fluorouracil + placebo | 20 | 3.3 | 7.4 |
| TPExtreme, Guigay 2021 | 541 | Cisplatin +5-fluorouracil + cetuximab | 57 | 6.2 | 13.4 |
| | | Cisplatin + docetaxel + cetuximab | 58 | 6.0 | 14.5 |

*ORR* overall response rate, *PFS* progression-free survival, *OS* overall survival
[a]Significant difference

advancements, no predictive biomarker could be identified, since neither EGFR expression by immunohistochemistry nor EGFR copy number by fluorescence in situ hybridization (FISH) did significantly correlate with cetuximab treatment outcomes [31, 32]. However, a recent observation might have identified a potential step forward in this matter. Based on a retrospective analysis of four phase II or III trials exploring different anti-EGFR targeted therapies in the R/M setting, a conclusion was drawn that objective responses were almost exclusively limited to patients with HPV-negative tumours compared with their HPV-positive counterparts, signalling a potential predictive role for HPV [33].

### *Immunotherapy*

Almost a decade passed before another breakthrough discovery was made, this time in immunotherapy using immune checkpoint inhibitors. Nivolumab and pembrolizumab, both targeting programmed cell death receptor 1, have become the first standard of care treatment in the second-line palliative setting, and their success has not been reproduced by other drugs so far (Table 20.2) [15, 16, 34, 35]. Comparing nivolumab with the investigator's choice, the landmark Checkmate-141 trial established a blueprint for expected immunotherapy toxicity, which was clearly more favourable than that of the standard of care (Table 20.3). Moreover, quality of life measured by physical, role, and social functioning, pain, sensory problems, and social-contact problems as well as symptom burden significantly improved in the experimental arm with nivolumab [15].

A logical consequence was the high anticipation of a randomized phase III trial for the first line setting, with the standard comparator being the EXTREME regimen. The results of the Keynote-048 trial exploring pembrolizumab with or without platinum/5-fluorouracil led to another breakthrough in this field because the experimental regimen proved to be statistically superior to EXTREME, not only in terms of median OS but with a survival benefit which seems to continue at long-term follow-up. This trial also introduced a long-awaited predictive biomarker for the first-line setting. The combined positive score (CPS) categorized cases into three major groups involving CPS $< 1$, CPS $\geq 1$, and CPS $\geq 20$. While no benefit of immunotherapy was noted in the lowest expressors, patients harbouring CPS-positive tumours had significantly longer estimate of median OS when treated with pembrolizumab/chemotherapy combination (13.6 vs. 10.4 months, $p < 0.0001$), and those with tumours expressing CPS at least 20 could derive such benefit even with single-agent pembrolizumab (14.9 vs. 10.7 months, $p = 0.0007$) [10, 36]. Again, the toxicity profile clearly favoured pembrolizumab monotherapy with any grade treatment-related adverse events appearing in 58.3% of patients (vs. 96.9% with EXTREME), grade 3 or more adverse events in 16.7% (vs. 69%, respectively), and adverse events leading to death in 1% (vs. 2.8%, respectively) [10].

**Table 20.2** Randomized trials with immune checkpoint inhibitors in recurrent and/or metastatic squamous cell carcinoma of the head and neck beyond the first line [15, 16, 35]

| Study, First Author and Year of Publication | N | Regimen | ORR (%) | median PFS (months) | Overall survival | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | at 12-months (%) | median (months) | hazard ratio (95% confidence interval) | p-value |
| CheckMate-141, Ferris 2016 | 361 | Nivolumab | 13.3 | 2.0 | 36 | 7.5 | 0.68 (0.54–0.86) | 0.01 |
| | | Control (methotrexate, docetaxel, or cetuximab) | 5.2 | 2.3 | 16.6 | 5.1 | | |
| Keynote-040, Cohen 2019 | 495 | Pembrolizumab | 14.5 | 2.3 | 37.3 | 8.4 | 0.80 (0.65–0.98) | 0.016 |
| | | Control (methotrexate, docetaxel, or cetuximab) | 10.1 | 2.1 | 27.2 | 6.9 | | |
| EAGLE, Ferris 2020 | 489 | Durvalumab | 17.9 | 2.1 | 37.0 | 7.6 | 0.88 (0.72–1.08) | 0.20 |
| | | Control (taxane, fluoropyrimidine, or cetuximab) | 17.3 | 3.7 | 30.5 | 8.3 | | |

*ORR* overall response rate, *PFS* progression-free survival

**Table 20.3** Treatment-related adverse events in the CheckMate-141 trial [15]

| | Nivolumab | | Investigator's choice[a] | |
|---|---|---|---|---|
| | n = 236 | | n = 111 | |
| Adverse events | Any grade (%) | G3–4 (%) | Any grade (%) | G3–4 (%) |
| Any (in ≥10% of patients) | 58.9 | 13.1 | 77.5 | 35.1 |
| **Selected TRAEs** | | | | |
| Skin | 15.7 | 0.0 | 12.6 | 1.8 |
| Endocrine | 7.6 | 0.4 | 0.9 | 0.0 |
| Gastrointestinal | 6.8 | 0.0 | 14.4 | 1.8 |
| Hepatic | 2.1 | 0.8 | 3.6 | 0.9 |
| Pulmonary | 2.1 | 0.8 | 0.9 | 0.0 |
| Hypersensitivity or infusion reactions | 1.3 | 0.0 | 1.8 | 0.9 |
| Renal | 0.4 | 0.0 | 1.8 | 0.9 |

*TRAEs* treatment related adverse events, *G* grade
[a]Methotrexate, docetaxel or cetuximab

### *Summary of Gains in the Recurrent and/or Metastatic Setting*

Cytotoxic chemotherapy typically yields a median OS of around 6 months in the first-line, with less than 5% of patients being alive at 5 years. Adding cetuximab to the platinum/5-fluorouracil combination nearly doubled the overall response rate from 20% to 36% and improved the median OS to 10 months, thus gaining almost 3 additional months, albeit long-term survivorship remained rare. Of note, despite high rates of severe acute toxicity, the quality of life was not compromised with the three-drug regimen. The third major step forward has been the introduction of immune checkpoint inhibitors. These agents provide only modest response rates in monotherapy, but patients who respond can maintain response for up to 2 years or more. Both in the first and later treatment lines, median survival gains have been in the range of 2 months. Importantly, the beneficial effects of single-agent immunotherapy are reflected in better quality of life and lower toxicity compared to chemotherapy-based regimens. From this perspective, it paves the way for more treatment options for elderly and less fit patients and, therefore, might help overcome their disproportionately low participation rate in clinical trials.

## Milestones in Non-surgical Treatment of Locoregionally Advanced Squamous Cell Carcinoma of the Head and Neck

In this setting, advancements have been shaped by discoveries in radiotherapy, paralleled by those in the field of systemic treatment. Since the introduction of orthovoltage radiotherapy in the 1920s, further progress has occurred almost regularly every 20–30 years, including the development of linear accelerators, telecobalt units, altered fractionation, and Intensity-Modulated Radiation Therapy (IMRT).

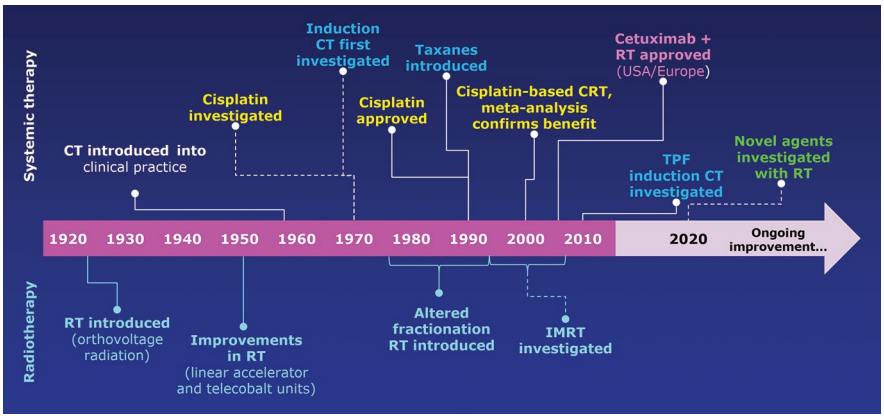


**Fig. 20.2** Development of non-surgical treatment of locoregionally advanced squamous cell carcinoma of the head and neck [37–42]. *RT* radiotherapy, *CRT* chemoradiation, *IMRT* intensity modulated radiotherapy, *TPF* taxane + platinum +5-fluorouracil

The latter has been evolving since the 1960s, approximately 40 years after the introduction of orthovoltage radiotherapy. Cisplatin was among the first active drugs in head and neck cancer and remained relevant to the present. Another major turning point was the integration of taxanes into treatment schedules in the early 1990s, followed by cetuximab 15 years later. Early attempts to optimize the use of systemic treatment focused on its administration in the neoadjuvant setting. The so-called "induction" chemotherapy has never been unequivocally proven to outperform the later-developed chemotherapy concurrent with radiotherapy but rather has become a subject of recurring academic debates. Nevertheless, if induction chemotherapy is opted for, the choice should fall on the TPF (docetaxel, cisplatin, 5-fluorouracil) regimen, according to the large phase III trials TAX 323 and TAX 324, the benefits of which were maintained even at long-term follow-up. However, as alluded to before, concurrent administration of chemotherapy, particularly single-agent cisplatin, in parallel with radiation, leads to the best efficacy outcomes, as confirmed by several large prospective randomized trials and meta-analyses (Fig. 20.2) [37–47].

## *Induction Chemotherapy*

In the early 1980s, clinical studies reported high response rates of induction chemotherapy with overall response rates between 85% and 90% and complete response rates between 35% and 55%. In addition, half of clinical complete responses were pathologically confirmed [48, 49]. Another noteworthy observation, as shown by Ensley already in 1984, was that chemotherapy could predict radiosensitivity. In a cohort of 60 treated patients, 42 achieved an objective response after induction chemotherapy, and 97% of these 42 reached complete response following subsequent

radiotherapy. Contrastingly, only 6% (i.e., 1 patient) of the remaining 18 patients, who did not respond to induction chemotherapy, achieved a complete response [50]. Following these early studies, several other randomized trials employing platinum-based induction regimens were conducted. The majority of them included operable cases and showed beneficial effect of induction chemotherapy in reducing the rate of distant metastases but not on OS, which hindered the universal adoption of this approach in clinical practice [51, 52]. Consequently, the question as to whether induction chemotherapy should be used in routine practice or not has not been resolved, yet the conundrum of selecting the optimal chemotherapy regimen has found its winner. In 2007, the discussion on induction chemotherapy was revisited with the publication of two independent studies, TAX 324 conducted in resectable and unresectable cases and TAX 323 only in unresectable cases. Both studies explored the docetaxel, cisplatin, and 5-fluorouracil combination relative to the platinum/5-fluorouracil doublet. They demonstrated a significant decrease in the hazard ratio for death with a 30% and 27% risk reduction, respectively, reflected in better PFS and OS [41, 42]. Long-term results confirmed sustained survival advantage [53, 54]. The benefit of the TPF regimen was also noted in the toxicity profile with less severe thrombocytopenia, nausea, vomiting, stomatitis, hearing loss, and toxic death than what was observed with the PF regimen [41, 42]. It improved short-term health-related quality of life and symptom control and proved to be cost-effective [55, 56]. Moreover, a significantly higher overall response rate was achieved in a larynx preservation protocol (80% vs. 59%) [57].

## *Concurrent Chemoradiotherapy*

Timing of chemotherapy in relation to radiotherapy plays a major role in treatment efficacy, and a comprehensive meta-analysis of individual data from 19,805 HNSCC patients treated with various schedules found that only concomitant administration of chemotherapy and radiotherapy leads to better OS with a 5-year and 10-year absolute benefits of 6.5% and 3.6%, respectively [47]. Among various anticancer agents, cisplatin has emerged as one of the most potent drugs. Compared with radiotherapy alone, single-agent cisplatin-based chemoradiotherapy enhances local control and/or OS, whether given as definitive treatment or adjuvantly after surgery [58]. According to four large randomized trials, three cycles of high-dose cisplatin (100 mg/m$^2$) administered every three weeks concurrently with conventionally fractionated external beam radiotherapy represent the standard of care [43–46]. In the adjuvant setting, extracapsular extension and/or positive resection margins with microscopic involvement at 5 mm or less were identified as the strongest negative prognostic factors and have subsequently been adopted in clinical practice as predictors to select patients for cisplatin-based chemoradiotherapy following curative-intent surgery [59].

Estimated 5-year OS rates range between 40% in the definitive setting and 50% in the postoperative setting. In addition to these still unsatisfactory efficacy

outcomes, treatment-related adverse events continue to raise concerns, both in the acute phase mainly with myelotoxicity, mucositis, and dysphagia and in the late phase with pharyngeal and laryngeal dysfunctions and feeding-tube dependence [60–62]. Given that acute side effects are often linked to peak levels in the blood, prolonged infusions or splitting high-dose to low-dose cisplatin may be beneficial in some clinical scenarios, such as in elderly patients or those with relative contraindications to cisplatin related to preexisting mild organ dysfunctions [63–65]. Other strategies to mitigate toxicity outcomes while preserving sufficient efficacy have relied on replacing cisplatin with other drugs. Among them, cetuximab garnered major attention after a 2006 publication of a randomized trial where it significantly reduced mortality and improved locoregional control when combined with radiotherapy, relative to radiotherapy alone [17, 66]. However, as mentioned earlier, further experience with cetuximab in patients with locoregionally advanced HNSCC has rather been disappointing, particularly in patients with HPV-positive oropharyngeal carcinoma, where substantially higher rates of locoregional failure were reported in three de-escalation trials in treatment arms with cetuximab compared with cisplatin administered either weekly- or three-weekly. Nevertheless, at the same time, these latter trials also confirmed the efficacy of concurrent cisplatin/radiotherapy in HPV-positive oropharyngeal carcinoma showing high OS rates with a 5-year estimate surpassing 80% [21–23].

### *Immunotherapy*

Following their success in the recurrent and/or metastatic setting, immune checkpoint inhibitors (e.g., pembrolizumab, avelumab) have been tested in several phase II and III randomized trials in the locoregionally advanced setting. Four completed studies explored concomitant administration of immune checkpoint inhibitors with definitive radiotherapy. They included PembroRad (GORTEC 2015–01) comparing pembrolizumab with radiotherapy and cetuximab with radiotherapy; JAVELIN Head and Neck 100 evaluating avelumab given prior to, concomitantly with, and for 1 year after radiotherapy vs. standard cisplatin-based chemoradiotherapy with placebo; GORTEC-REACH assessing avelumab with cetuximab and radiotherapy followed by avelumab for 1 year vs. cisplatin-based radiotherapy in fit patients or cetuximab-based radiotherapy in unfit patients; and Keynote-412 comparing pembrolizumab with cisplatin-based chemoradiotherapy vs. placebo with cisplatin-based chemoradiotherapy [67–70]. Possibly influenced by the inclusion of lymph nodes, as key organs of immunogenesis, in radiation fields, none of these trials met their primary efficacy endpoint, thus mitigating further attempts at concomitant administration of immune checkpoint inhibitors with radiotherapy. Despite these failures, recent results suggest promising outcomes with neoadjuvant and adjuvant administration of immune checkpoint inhibitors with respect to radiotherapy. For instance, neoadjuvant pembrolizumab with platinum and docetaxel in stage II-III laryngeal carcinoma resulted in a pathologic complete response in three quarters of

patients after four treatment cycles [71]. Another trial compared sequential vs. concurrent administration of pembrolizumab with chemoradiation and found significantly better locoregional control and a numerically better PFS and OS with the former approach [72].

## Conclusions

Over the past 50 years, the median OS in patients with R/M-HNSCC has nearly doubled, increasing from 7 months to 13 months or more. While long-term survival has been exceptional when using traditional cytotoxic drugs and EGFR inhibitors, this has changed for a small proportion of patients with the introduction of immune checkpoint inhibitors as illustrated by the so-called tail in the survival curve, showing a survival plateau at approximately 20% in an unselected population at around the 4-year landmark in the most recently published update of the Keynote-048 trial [73]. Nevertheless, the prognosis in the palliative setting remains poor for the majority of the patients, emphasizing the need to enrol patients in clinical trials exploring novel combinations. On the other hand, progress in the locoregionally advanced HNSCC with systemic therapy has been modest. The standard of care chemoradiotherapy schedule with cisplatin has remained unchanged for the past two decades as has been the approach to patients with HPV-positive oropharyngeal tumours. Successful integration of immunotherapy in the management of locoregionally advanced disease will be more cumbersome and will take longer than expected, yet it holds the promise of a great leap forward in the near future.

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# Chapter 21
# Therapeutic Potential of Antibody-Drug Conjugates in Head and Neck Cancers

**Daria Maria Filippini and Christophe Le Tourneau**

## Introduction

Head and neck cancer (HNC) is the seventh most common cancer worldwide with high mortality rates [1]. Head and neck squamous cell cancer (HNSCC) represents more than 90% of HNC cases and the rest comprises rare and various histologies including salivary gland cancers (SGCs). In the recurrent/metastatic (R/M) HNSCC, despite the recent introduction of immune checkpoint inhibitors, only a minority of patients benefit from these treatments and the prognosis remains still poor [2, 3]. Given these challenges, there is an urgent demand for innovative treatments to address the critical need in managing this disease. Nasopharyngeal tumor, when observed in non-endemic populations, and SGCs are rare malignancies that differ from HNSCC in several aspects, including their etiology and treatment approaches. Moreover, patients with these histotypes are typically not included in clinical trials of HNC and therefore clinical evidence is more difficult to obtain [4]. SGCs, encompassing more than 20 distinct tumor types, primarily rely on definitive surgical management as the mainstay of treatment followed by adjuvant radiotherapy based on histological and clinical features [5]. In metastatic nasopharyngeal cancer, besides palliative chemotherapy, immune checkpoint inhibitors represent a novel promising strategy in this setting while no standard second-line treatment exists [6, 7].

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The antibody drug-conjugates (ADCs) represent a novel therapeutic class that has been successfully developed for several indications, including hematologic and solid malignancies.

The more recent FDA approvals of ADCs concern the treatment of advanced triple negative breast cancer, human epidermal growth factor receptor 2 (HER2)-low breast cancer, cervical cancer and urothelial tumors [8–11]. For HNC, various ADCs are being explored in the R/M disease setting in previously treated populations with most of the available data coming from early phase trials.

In this chapter, we review the ADCs investigated for HNC including their mechanisms of action, efficacy, and tolerability.

## ADC: General Structure and Main Features

ADCs represent a category of anticancer drugs consisting of three essential elements: a monoclonal antibody (mAb) designed to target a specific antigen, a potent cytotoxic payload and a linker that connects the antibody to the payload (Fig. 21.1). Combining the selectivity offered by mAb and the potent effects of the payloads, the ADCs result in a "smart drug delivery" system capable to increase therapeutic efficacy while minimizing the toxicity of anticancer agents [12]. The specific features of each element distinguish the various ADCs. The antigen should have low/no shedding into the circulation, an elevated internalization and recycling capability (the latter refers to the possibility to re-exposition on the cell to link the antibody).

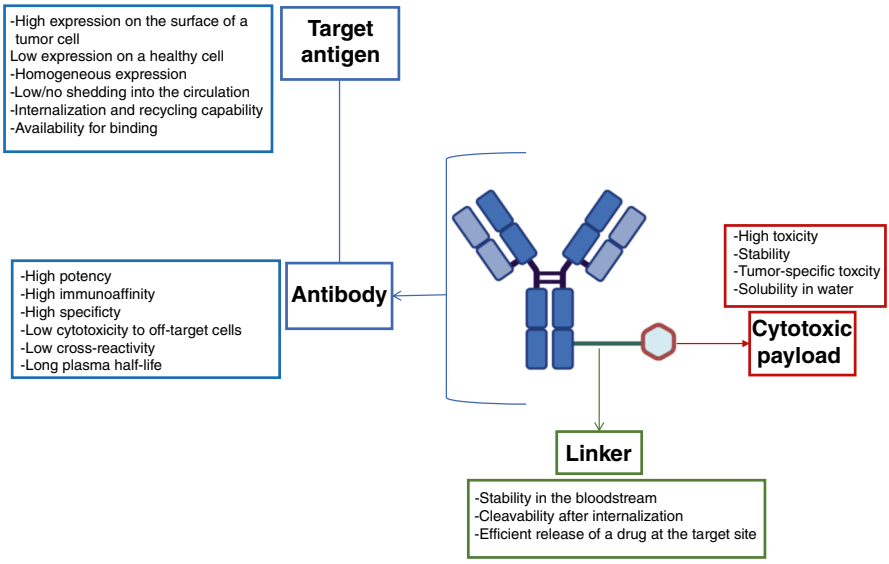


**Fig. 21.1** The ADC elements and expected features. (Created with Biorender.com)

The antibody is crafted to exhibit high potency, high specificity, high immunoaffinity, low cytotoxicity to off-target cells, a low cross-reactivity and a long-plasma half-life. Typically, the antibody involves a chimeric/humanized monoclonal IgG1 targeting a protein preferentially expressed on the tumor cell surface. The linker serves as a crucial element of the ADC connecting the antibody to the cytotoxic payload [13]. It should be stable in blood circulation and facilitate an effective release rate of the payload once internalized. Linkers can be cleavable or non-cleavable. Non-cleavable linkers (e.g., lysosomal degradation of mAb) enhance ADC stability, with the antibody, linker, and payload being internalized by the target cell for degradation before releasing the payload exclusively within the cell. This results in an efficient antitumor activity but lacks a bystander effect, meaning the payload is confined to tumor cells and does not affect surrounding cells [14]. Non-cleavable ADCs are most effective against cancers that have high and uniform expression of the target antigen. An example of an ADC utilizing this mechanism is ado-trastuzumab emtansine known for its efficacy in the treatment of breast cancer. On the other hand, cleavable linkers (e.g., acid/redox/lysosomal sensitive) enable payload release into the extracellular space, promoting the bystander effect [14]. This mechanism offers the additional advantage to be effective against heterogeneous expression of target antigens and their low-density. Examples of cleavable linkers are in trastuzumab deruxtecan, sacituzumab govitecan and enfortumab vedotin.

The connection of the linker to the antibody conditioned the drug-to-antibody-ratio (DAR) indicating the number of drug molecules bound to each ADC. DAR fluctuates considerably and is influenced by various other factors related to ADC composition. Additionally, the DAR values can be impacted by the site of conjugation and whether light or heavy conjugated chains are employed. A high DAR value is crucial for an increasing antitumor efficacy, even in cases where the density of antigens on tumor cells is low. This property enhances the bystander effect and it is observed in trastuzumab deruxtecan, sacituzumab govitecan and enfortumab vedotin [15].

The cytotoxic payload is characterized by a high tumor-specific toxicity, stability and solubility in water. Three key classes of payloads are represented in currently approved ADCs, including anti-microtubule agents, alkylating agents, and topoisomerase 1 inhibitors.

Potential adverse events associated with ADCs often stem from a premature release of the cytotoxic drug into the bloodstream before reaching the cancer cell.

The common severe toxicities of grade (G) 3/4 linked to ADCs align with the characteristics of their payload class [16]. For example, ADCs with monomethyl auristatin E (MMAE) frequently cause severe anemia, neutropenia and peripheral neuropathy. Emtansine (DM1) ADCs often lead to G3/4 thrombocytopenia and hepatic toxicity. Additionally, severe ocular toxicity is associated with ADCs containing monomethyl auristatin F (MMAF) and ravtansine (DM4). Infusion-related reactions (IRRs) can also occur during or after ADCs infusions.

Of note, resistance mechanisms to ADCs are emerging and encompass factors like diminished antigen expression, altered ADC internalization/trafficking,

dysregulation of apoptotic pathway, augmented drug efflux and modified internalization processes [17].

## ADC Targets in Head and Neck Cancers

The targets of ADCs evaluated in HNC include epidermal growth factor receptor (EGFR), HER2, trophoblast cell-surface antigen 2 (TROP2), integrin beta 6 (IB6), tissue factor (TF), CD44v6, CD166, CD71, Nectin-4 and p-cadherin highlighting their safety and efficacy results for HNC patients. Table 1 reports the main features and results of these ADCs.

## EGFR

EGFR is highly expressed in HNSCC, playing a crucial role in the carcinogenesis and tumor progression and is correlated with poor prognosis. Over the last two decades, EGFR targeting has been intensely pursued as a treatment strategy for metastatic HNSCC. One approach used monoclonal antibodies to inhibit the extracellular domain of EGFR, thus blocking natural ligands binding. Unfortunately, patients often develop resistance with consequent tumor growth and relapse. Mechanisms of acquired cancer resistance to anti-EGFR could develop either from sustained EGFR signaling or downstream molecular alterations or through crosstalk with related receptors like HER2 and HER3 [18]. Overcoming this resistance is crucial for enhancing patients' prognosis. Here, we discuss two anti-EGFR ADCs evaluated in clinical trials for HNC.

### *MRG003*

MRG003, a humanized anti-EGFR IgG1 antibody conjugated to the MMAE payload (a microtubule inhibitor) via a valine-citrulline linker. The preclinical data of MRG003 has not been published. Initial findings from a first-in-human phase I study demonstrate acceptable safety and promising activity. The study allowed prior treatment with anti-EGFR mAbs, and in the dose expansion cohort patient selection was based on EGFR expression [19]. The phase II study evaluated efficacy and safety of 67 R/M HNSCC categorized into two groups of MRG003 (35 and 32 patients for the dose of 2.0 and 2.3 mg/kg Q3W, respectively). Most patients had received prior platinum (95.5%), PD-1/L1 inhibitor (76.1%), anti-EGFR mAbs (47.8%).

In the 2.3 mg/kg subgroup the ORR was 43%.Interestingly, the immunohistochemistry detected EGFR positivity in 62 out of 65 patients (95.4%). Concerning

safety, the most common G3/4 treatment-related adverse events (TRAEs) were leukopenia, neutropenia, anemia, vomiting, anorexia, hypokalemia and nausea [20]. For the nasopharyngeal cohort, efficacy results were presented for both subgroups (2.0 mg/kg and 2.3 mg/kg) for a total of 61 patients reporting an ORR of 47% [21]. For the HNSCC cohort, MRG003 is under investigation compared with cetuximab or methotrexate in a randomized multicenter phase 3 trial (NCT05751512) involving patients with R/M disease after failure of anti-PD-1/L1 inhibitors and platinum-based therapy.

### *Losatuxizumab Vedotin*

Losatuxizumab vedotin (formerly ABBV-221) is an ADC designed to target EGFR, conjugated with the potent payload MMAE through depatuxizumab, a cathepsin cleavable valine-citrulline linker. It was evaluated in a first-in-human phase I trial where prior treatment with EGFR mAb was allowed. Of the five HNSCC patients included in this study one showed a partial response. Remarkably, this patient had been previously treated with cetuximab and showed increased levels of EGFR and EGFR ligands (amphiregulin, epiregulin). Notably, G3/4 TRAEs were reported in 60% of patients across all cohorts, with IRRs being the most prevalent. Due to the frequent occurrence of IRRs, the trial was prematurely closed, and the maximum tolerated dose was not determined. This suggests that the mAb component of the ADC may impose dose limitations [22].

## HER2

HER2, also identified as ERBB2, belongs to the EGFR family, and its overexpression is pivotal in the initiation and maintenance of various malignancies. In HNC, the HER2 overexpression is mainly found in salivary duct carcinoma (SDC) (up to 30%) and adenocarcinoma not otherwise specified (up to 21%) and is associated with poor prognosis [23]. Trastuzumab deruxtecan (T-DXd, DS-8201) is an ADC composed of a humanized anti-HER2 IgG1 monoclonal antibody, covalently linked to a topoisomerase I inhibitor (DXd) through a tetrapeptide based cleavable linker. T-DXd exhibits a drug-to-antibody ratio (DAR) of approximately 8 [24]. The released payload is membrane-permeable, enabling it to exert a cytotoxic effect on tumor cells proximal to the targeted cells, irrespective of HER2 expression levels. The elevated DAR and the bystander antitumor effect may contribute to the antitumor activity in cancers with heterogeneous HER-2 expression. In a pooled analysis of two open-label phase I trials of T-DXd [25], 17 patients with advanced unresectable and/or metastatic SGCs with HER2 expression that were refractory or intolerant to a prior systemic chemotherapy regimen or for which no standard treatment was available, were included. Most of patients (53%, n = 9) had SDC followed by

adenocarcinoma in 12% (n = 2). In 29% (n = 5) the carcinoma remained unspecified and for one patient histological data were not available. Among these 17 patients,14 had received prior HER2-targeted agents and 13 patients had undergone prior radiotherapy. The ORR was 59% and TRAEs were reported in 60% of cases with the most common being hematological toxicity. The interstitial lung disease (ILD) was observed in 29% (5/17) of patients in this analysis, with one patient experiencing G3 severity. However, no deaths attributable to ILD were reported. The findings from this pooled analysis offer evidence that clinical benefits can be achieved with T-DXd in patients with HER2-expressing advanced SGCs with manageable adverse events. Nevertheless, longer follow up is necessary to assess the impact of T-DXd on survival outcomes.

## TROP2

TROP2 is a transmembrane glycoprotein that was first discovered in human trophoblast and choriocarcinoma cell line and is highly expressed by many epithelial cancers [26]. Sacituzumab govitecan consists of a humanized anti-TROP2 monoclonal IgG1 linked to the cytotoxic payload, SN-38, which is the active metabolite of irinotecan and a topoisomerase 1 inhibitor, via a cleavable linker. This treatment has received approval for metastatic triple negative breast cancer and pretreated hormone-positive/HER2 negative metastatic breast cancer, and as a second line treatment for metastatic urothelial cancer [8, 27]. Recognizing the high expression of TROP2 in HNSCC, sacituzumab govitecan was evaluated in a phase II basket trial, TROPiCS-03 (NCT03964727), including HNSCC. The trial included 43 HNSCC patients, revealing an ORR of 16% and a 6-month duration of response (DOR) of 43%. Notably, G3/4 TRAEs were reported in 44%, with the most prevalent being neutropenia, leukopenia, anemia, and nausea [28]. Although evaluated in a heavily pretreated population, the observed efficacy of sacituzumab govitecan efficacy in HNSCC appears less remarkable compared to other cancers. These initial findings underscore the need for further investigation into the efficacy of sacituzumab govitecan as a potential treatment for HNSCC.

## Integrin-Beta 6 (IB6)

IB6 is prominently expressed in HNSCC, playing a substantial role in tumor pathogenesis and invasiveness; its upregulation correlate with a poor prognosis [29]. The humanized anti-IB6 antibody, SGN-B6A (h2A2), is linked to a MMAE payload via a cleavable valine-citrulline linker. Preliminary data from the SGNB6A-001 (NCT04389632) phase I dose escalation/expansion study, which enrolled 62 HNSCC (28%) out of 220 patients, indicated an ORR of 23%. Across all cohorts, G3 or higher TRAEs were reported in 20% of cases. Notably, all HNSCC patients

had received prior systemic therapy (previous treatment with anti-PD1 agents was allowed), with 86% undergoing at least two lines of therapy for the R/M setting [30]. SGN-B6A exhibited manageable tolerability and promising preliminary antitumor activity.

## Tissue Factor (TF)

TF is known for its role in promoting tumor growth, angiogenesis, and metastasis. TF is highly expressed in HNSCC and correlates with a poor prognosis. This transmembrane glycoprotein plays a pivotal role in the physiological initiation of the blood coagulation cascade [31]. Tisotumab vedotin (TV), a human anti-TF IgG1-kappa antibody linked with a MMAE payload and a cleavable valine-citrulline linker, has obtained approval for the treatment of advanced or recurrent cervical cancer with progression on or after chemotherapy [10]. Intriguingly, HNSCC exhibits TF expression in similar proportion to cervical cancer. The open-label phase II multicenter study, InnovaTV 207 (NCT03485209), evaluated TV at 1.75 mg/kg administered every 2 weeks for advanced tumors, including R/M HNSCC. Eligible patients could have received up to 3 lines of systemic therapy for R/M setting and must have received prior platinum-based therapy and an immune checkpoint inhibitor, if eligible.

Fifteen HNSCC patients were included, revealing an ORR of 40%. The TRAEs higher or equal to G3 were reported in 27% of cases. Most common TRAEs included dry eye, keratitis and fatigue. Eleven patients (73%) had received a dose modification due to AEs and two patients discontinued treatment due to peripheral sensory neuropathy and dry eye [32]. These findings suggest that TV may be a promising treatment option, with encouraging antitumor activity at a higher dose administered Q2W, while maintaining an acceptable safety profile in HNSCC, especially after prior platinum and immunotherapy combination.

## CD44v6

CD44v6, a tumor-associated antigen abundantly expressed in HNSCC, lacks tumor selectivity, being also expressed in normal squamous epithelium, including skin keratinocytes [33]. Bivatuzumab mertansine (BIWI 1) is an immunoconjugate consisting of mertansine (DM1), a highly potent antimicrotubule agent coupled to a IgG1kappa mAb against CD44v6, which is strongly expressed by more than 95% of HNC, mostly pharyngeal and laryngeal cancers, and is related to advanced stage and poor prognosis [34]. The available data from a first-in-human phase I trial involving 32 HNSCC cases, irrespective of CD44v6 expression, indicated an ORR of 10% (3/31 evaluable patients). Notably, G3/4 TRAEs were observed in 25% of HNSCC patients, with skin reactions in 80% [35]. One death attributed to toxic

epidermal necrolysis emphasizes the need for a careful management while targeting antigens highly expressed in normal tissues.

## CD166

CD166 is implicated in angiogenesis, inflammation, tumor propagation, invasiveness, and various immune processes [36]. Praluzatamab ravtansine (CX-2009), is a CD166-targeting probody drug conjugate. In this innovative approach, the monoclonal antibody is conjugated to the microtubule inhibitor (DM4), via a disulfide cleavable linker, with an average DAR of approximately 3.5 for the conjugated species. Safety and activity data derive from a first-in-human phase I/II trial, including nine HNSCC patients out of 99 included patients (9%). No selection based on CD166 was used. The ORR was of 11%, and G3/4 TRAEs were reported in 37% across all cohorts (the most common were ocular toxicity, keratitis, liver enzyme increase, and peripheral neuropathy) [37]. The typical ocular adverse event underscores the influence of the anticancer drug on the safety profile of ADCs, indeed DM4-conjugated ADCs are often associated with ocular toxicity. While preliminary results indicate acceptable toxicity and signal of anticancer activity in patients with advanced cancers, further exploration is warranted.

## CD71

CD71, known as transferrin receptor 1, is widely expressed on normal cells and plays a crucial role in cellular iron uptake [38]. CX2029, a CD71-targeting Protease-Activatable Antibody Prodrug Conjugate, consists of a protease-activatable antibody prodrug targeting CD71, conjugated to MMAE via a cysteine protease-cleavable dipeptide linker (valine–citrulline), with a DAR of 2. Tested in a first-in-human phase I/II trial, it was evaluated irrespectively of CD71 expression. Of the 45 enrolled patients, 8 patients (18%) were affected by HNSCC, reporting an ORR of 12% in this cohort and G3/4 TRAEs across all cohorts of 60%, mainly involving hematologic toxicity, occurring in a dose-dependent manner whereas IRRs were not dose dependent and mostly G1/2 [39]. These results highlight preliminary evidence of the tolerability and clinical activity of CX-2029 monotherapy in previously treated patients.

## Nectin-4

Nectin-4 is an immunoglobulin-like transmembrane protein physiologically involved in the Ca2+-independent formation of adherens junctions and tight junctions between cells as well as in cell movement and survival and it is prevalent in HNSCC

[40]. Enfortumab vedotin is an ADC composed of a fully human anti-Nectin-4 IgG1 kappa monoclonal antibody conjugated to MMAE by a protease-cleavable linker. Already approved as single agent in advanced, treatment-refractory urothelial cancer [11], enfortumab vedotin showed promising results in a multicohort, open-label phase II study (EV-202) involving 46 HNSCC patients. For HNSCC patients, the trial reported ORR of 24%, and G3/4 TRAEs in 35% of cases, with skin reactions and peripheral neuropathy being the most common. The safety profile aligns with observations in urothelial carcinoma patients, presenting no new safety signals [41]. Enfortumab vedotin demonstrates encouraging activity in a heavily pre-treated HNSCC population and a study investigating the combination of enfortumab vedotin with pembrolizumab is ongoing, also for HNSCC cohort (NCT04225117).

## P-Cadherin

P-cadherin facilitates calcium-dependent cell-to-cell contact across junctions of the epithelium [42]. It represents the target of PCA062, a first-in-class ADC comprising of a human IgG1 monoclonal antibody conjugated to a non-cleavable maytansine-derived SMCC-DM1 linker-payload. PCA062 was evaluated in a first-in-human phase I trial enrolling 47 patients with HNSCC, triple-negative breast cancer and esophageal cancer (squamous and adenocarcinoma). Of the 6 included HNSCC patients, 1 patient experienced partial response (17%); across all cohorts, G3/4 TRAEs were reported in 28% of cases, predominantly involving AST increase, thrombocytopenia and anemia [43]. This trial underscores that non-cleavable linkers minimize off-target toxicities but may narrow the therapeutic window.

## Conclusions and Future Directions

The advent of ADCs addresses the pressing need for new treatments in HNC, including rarer histotypes. The significant interest of ADCs lies in combining the precision of antibodies with the potency of payloads. This approach has transformed the treatment of other cancers such as breast, cervical and urothelial tumors.

For HNC, while intriguing efficacy results have emerged from some early phase clinical trials investigating ADCs mainly in selected patients overexpressing a specified target, the duration of response is still unknown. Current and future research is essential to identify clinical applications in this peculiar population. Longer follow-up of ongoing clinical trials and a higher number of HNC patients included, mainly for rarer histotypes should be considered.

Moreover, the future research will have to tackle numerous challenges posed by the potential introduction of ADCs in HNC, such as the necessity to identify biomarkers for predicting response, a deeper understanding of ADCs immunomodulatory properties, and the best sequences and/or combinations of such therapies.

In conclusion, the ADCs are poised to emerge as a new standard of care for recurrent/metastatic HNC, likely as a backbone for combination therapies; however, further investigations including sufficiently powered randomized trials are required to fully understand their potential for survival outcomes.

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# Chapter 22
# Intratumoral Drug Administration: More Potential in Head and Neck Cancer?


**Kevin J. Harrington**

## Introduction

Head and neck squamous cell carcinoma (HNSCC) represents a varied set of disease presentations affecting multiple sites within the head and neck region. These include the oral cavity, pharynx, larynx, nasal cavity, and paranasal sinuses [1]. These diagnoses are a major global health challenge, not least because they are relatively more common in less developed countries. Data from The Global Cancer Observatory (GLOBOCAN) in 2020 reported that HNSCC ranked as the seventh commonest cancer worldwide. Alarmingly, future projections up to 2030 suggest a dramatic global increase, mainly attributable to tumors associated with the human papillomavirus (HPV) [1].

Despite knowledge that outcomes from head and neck (and other) cancers are related to stage at diagnosis, the majority (approximately 60%) of patients are still diagnosed with advanced stage III and IV disease. Even with aggressive local and systemic multimodality therapies, rates of persistent and/or recurrent disease remain as high as 40–60% and most of these patients will not be cured of their disease. For patients with distant metastasis, the outlook is dismal with 5-year overall survival rates no greater than 20% with current state-of-the-art systemic therapies (first-line single-agent immunotherapy or immunotherapy-chemotherapy combinations followed by subsequent second- and third-line treatments) [2].

HNSCC has a distinct pattern of locoregional disease recurrence around the primary site, tumor bed and regional lymph nodes. Disease recurs locoregionally in 15–50% of patients with HNSCC. The fact that most of these recurrences arise in areas that have already been treated with surgery and/or radiotherapy severely limits options for subsequent salvage [3] and attempts at local therapies are frequently

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associated with high levels of morbidity and treatment failure. At present, most patients who present with loco-regional recurrence as an isolated site of disease, or with such recurrence as part of a more widespread process in association with distant metastatic disease, will receive systemic, rather than locally administered, therapies.

The management of recurrent or metastatic HNSCC cases not amenable to curative-intent, local therapies is a significant challenge [4]. Since the 1970s, in common with other solid cancers, HNSCCs have been treated with oral or, more commonly, intravenous cytotoxic drugs. Systemic treatment administration enables drug distribution to all tumor sites, but it also increases the risk of normal tissue side effects [5]. This is a major consideration, given the fact that patients with HNSCC tend to have high levels of comorbidity, especially in regard to cardiovascular, respiratory, renal and hepatic diseases. All of these factors can be associated with an increased risk of systemic toxicities and narrow the therapeutic index of conventional cytotoxic agents [6]. Patients often require reduction in the doses and scheduled frequency of administration of systemic cytotoxic agents and this can adversely affect the likely benefit of treatment. Some head and neck cancers may be relatively poorly targeted by systemically administered agents, not least because poorly vascularized lesions [7] with ulceration are associated with low drug penetration given their local necrosis, irregular vascularization, co-existing bacterial colonization and immunosuppressive tumor microenvironment.

All of these come into play when considering the potential of using the intratumoral route as a means of treatment administration. Intratumoral therapy aims to deliver the antitumor agent directly into one or more malignant lesions, with the goal of distributing the therapy as widely as possible across the disease site. Most therapeutic approaches aim to induce tumor death *in situ*, releasing tumor antigens, and locally activating components of the immune system to trigger an antitumor response (*in situ* vaccination) at the injected site and in locoregionally-located, draining lymph nodes. Ideally, this response will be accompanied by a more generalized systemic immune response, leading to responses at non-injected locations. This latter event is often referred to as the "abscopal effect [*ab*—away from, *scopus*—target]", but use of this term may be inappropriate if the intratumoral therapy is given concomitantly with systemic therapy because no tumor site is truly abscopal in such circumstances [8–10]. Alternative terminology, focusing on the primary importance of the distinction between injected and non-injected lesions, makes use of the terms enestic (injected) and anenestic (uninjected) [7]. In addition to their goal of achieving direct, local effects, intratumoral therapies seek to reduce the burden of systemic exposure, and the inevitable systemic toxicities, arising from oral or intravenous drug administration. In addition, for certain therapeutic agents, systemic administration may be far from ideal. For example, large macromolecules or biological therapies (liposomes, viruses, bacteria) may be cleared rapidly, with very high first-pass kinetics, by elements of the patient's fixed-tissue macrophage or reticuloendothelial system in the liver, spleen and lung. For such agents, direct intratumoral administration represents an efficient and, potentially, effective means of drug delivery.

For patients with locoregionally recurrent HNSCC, disease is very often directly accessible for intratumoral injection. Available sites can include points of mucosal recurrence, especially oral and nasal cavity and parts of the pharynx that can be accessed under direct vision. Certainly, other mucosal sites might be considered for direct intratumoral injection under sedation/general anesthesia or by radiologically-guided procedures, but these are less attractive given the logistical, ethical and medical challenges presented by needing to give regular, repeated doses of therapeutic agents. The greatest number of patients with recurrent HNSCC who are considered for intratumoral therapy approaches are those with cervical lymph node, subcutaneous and cutaneous disease. Such sites are eminently injectable, although there may be significant safety concerns in some patients in regard to the airway and neurovascular bundle in the neck. In this respect, consideration needs to be given to the risk of post-injection swelling/inflammation that might be associated with airway compromise, vascular insufficiency, bleeding or nerve compression (Fig. 22.1).


**Fig. 22.1** Intratumoral injections in head and neck cancer. The site of nodal disease in right level 3 is easily accessible for direct intratumoral injection (indicated by the large red arrow), either freehand in the clinic or under image-guidance in the interventional radiological suite. The proximity of disease to the patient's airway and the neurovascular space in the neck represents a cause for concern in view of the potential risks associated with post-treatment swelling or inflammation. In this case, there would be significant risks associated with an already compromised airway that would, almost certainly, necessitate insertion of a prophylactic tracheostomy. In addition, concerns about vascular/neural compromise or, more likely, vessel compromise/bleeding would require great caution. Attention must also be paid to other factors that might increase the risk of bleeding (e.g. concomitant medication with anti-platelet agents or anti-coagulants and/or disease- or treatment-induced thrombocytopenia)

## Potential Therapeutic Agents

A host of directly administered agents might be considered for intratumoral therapy approaches. For the purposes of this review, the discussion will be restricted to 3 classes of agents: oncolytic viruses (specifically herpes simplex virus); genetically-modified bacteria (*Yersinia enterocolitica*) and a naturally-sourced, novel diterpene ester (tigilanol tiglate).

### *Oncolytic Virotherapy*

Oncolytic virotherapies (OVs) can be broadly characterized based on virus type (DNA or RNA), genome size, tropism, mechanism of cellular entry, and their tendency to induce anti-viral immune responses (immunogenicity). They are either native (so-called wild-type viruses) or have been genetically modified to enable them to enter, replicate in, and kill tumors without damaging locoregional or distant normal tissues [11].

Local tumor killing by OVs leads to release of tumor-associated antigens (hopefully including tumor neoantigens), viral pathogen-associated molecular patterns (PAMPs), and cell-derived, pro-inflammatory damage-associated molecular patterns (DAMPs), that are needed for the induction of an effective systemic anti-tumor immune response [12, 13]. A simplified schema of the mechanism of action of many OVs is depicted in Fig. 22.2.

Many OVs can be genetically manipulated using recombinant techniques to enable them to carry transgenes, the expression of which can result in activation of innate and adaptive immunity by triggering the production of cytokines, antibodies capable of binding to immune checkpoint molecules, and/or other immune stimulatory proteins locally in the tumor microenvironment [13]. Direct killing of tumor cells within the injected lesion can lead to recruitment and activation of professional antigen-presenting cells (e.g. dendritic cells) within the tumor microenvironment. This, in turn, can lead to their being able to ingest debris associated with tumor lysis and this can include both viral and tumor-associated antigens (TAA). These dendritic cells become activated and they begin to express surface markers (e.g. CD80, CD86 and CCR7) that are associated with their being able to migrate to loco-regional lymph nodes where they are able to interact with populations of T cells. Dendritic cells can present externally-derived TAA in the context of their own major histocompatibility complex (MHC) molecules: through MHC class I molecules, they can present to cytotoxic CD8+ T cells; through MHC class II molecules, they can present to CD4+ T cells. If the dendritic cell finds T cells (CD4+ and CD8+) with T cell receptors (TCR) that recognise the TAA, they are capable of driving huge expansion and activation of T cells that multiply within the loco-regional lymph nodes before entering the bloodstream and seeking sites of disease in which the TAA is expressed. Such populations of T cells are, therefore, capable of finding

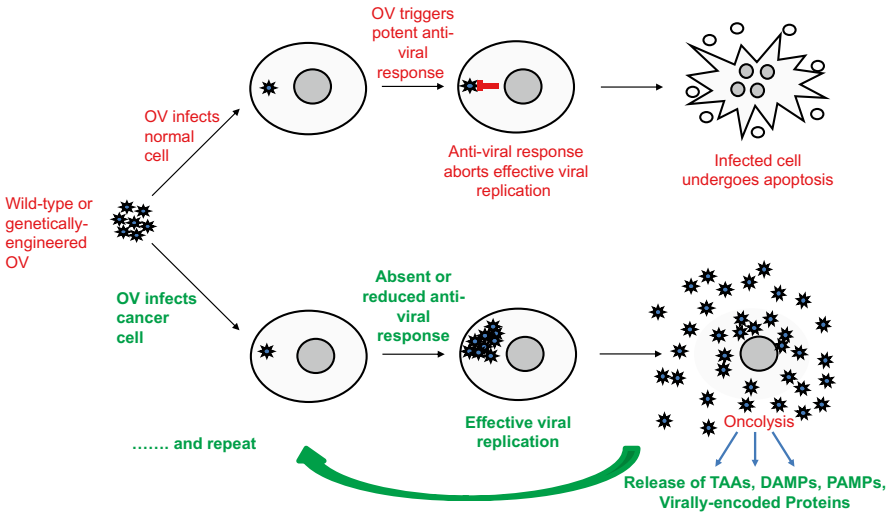


**Fig. 22.2** Schema for mechanism of action of oncolytic virotherapies. When a wild-type or genetically-engineered oncolytic virus infects a normal, non-cancerous cell, it triggers potent innate anti-viral immune responses that effectively abort viral replication and, at the same time, engage pathways that lead to immunologically-silent apoptosis of the infected cell. As a result, there is no productive viral infection. In contrast, if an oncolytic virus infects a cancer cell, it is frequently able to replicate effectively because cancer cells have often lost/mutated normal innate, anti-viral mechanisms. As a consequence, the virus can grow and rupture (oncolyse) the cell, leading to release of tumor-associated antigens (TAA), danger-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs) and virally-encoded proteins (e.g. cytokines, antibody-like immune checkpoint inhibitors)

and engaging tumor cells both in the original, loco-regional, injected (enestic) site but also at local/distant uninjected (anenestic) sites where they can mediate so-called abscopal responses (Fig. 22.3).

The ability of OVs to increase the immunological heat in so-called "cold" tumor microenvironments, in association with their abilities to increase the recruitment of a range of different immune cells, including CD8+ cytotoxic T cells, has been seen as a means of enhancing the susceptibility of tumors to immune checkpoint inhibitors (ICIs). Such approaches have found favor in a number of studies, given the relative lack of efficacy of ICIs alone in non–immune-infiltrated tumors [14].

Talimogene laherparepvec (T-VEC), a herpes simplex virus type 1 (HSV-1) which encodes granulocyte-macrophage colony-stimulating factor (GM-CSF), was designed to enhance anti-tumor immune response in addition to its direct oncolytic effect [5]. T-VEC was approved by the US Food and Drug Administration (FDA) in 2015 for treatment of advanced and recurrent melanoma [15]. The genetic basis of the anti-cancer selectivity of T-VEC is described in Fig. 22.4. In the context of head and neck cancer, studies have evaluated its efficacy in both curative and recurrent/metastatic (R/M) settings. In the curative setting, 17 patients with node-positive, stage III/IV HNSCC were treated with chemoradiotherapy (70 Gy/35 fractions with

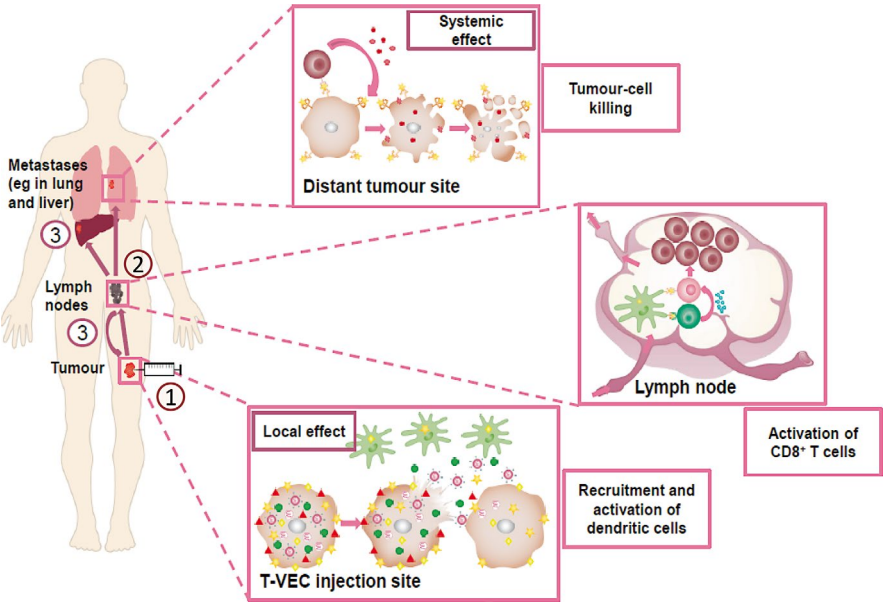


**Fig. 22.3** Schema for direct and indirect, immune-mediated actions of T-VEC oncolytic virotherapy (depicted here for deposit of malignant melanoma in the leg). (1) Local effect. Direct intratumoral injection of virus leads to cancer cell infection, viral replication and subsequent oncolysis in injected (enestic) lesion. Dying tumor cells release tumor-associated antigens (TAA), danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), in addition to encoded proteins (e.g GM-CSF). These attract and activate dendritic cells (spiky green cells) which become mature and migrate to local lymph nodes bearing TAA components. (2) Lymph node. Dendritic cells activate CD4+ and CD8+ T cells which amplify and enter the systemic circulation. (3) Distant tumor site. Activated T cells migrate to original site of injection, but also to uninjected disease in the lung and liver, where they are able to mediate additional, indirect tumor cell killing

concomitant cisplatin 100 mg/m$^2$ on days 1, 22, and 43) and dose-escalating T-VEC by intratumoral injection on days 1, 22, 43, and 64 [16]. Patients completed treatment without delays to chemoradiotherapy or dose-limiting toxicities. Fourteen patients (82.3%) showed tumor response by Response Evaluation Criteria in Solid Tumors (RECIST), and pathologic complete remission was confirmed in 93% of patients at pre-planned neck dissection. T-VEC was detected in injected and adjacent uninjected tumors at levels higher than the input dose, indicating viral replication. No patient developed locoregional recurrence, and disease-specific survival was 82.4% at a median follow-up of 29 months (range, 19–40 months). In the palliative context, T-VEC has been tested in a combination with pembrolizumab in the MASTERKEY-232 study [17]. Rather disappointingly, an overall response rate (ORR) of only 13% was observed, with an increased ORR of 18% in the PD-L1-positive cohort. These data were not sufficiently different to data for pembrolizumab alone from historical control populations to justify initiation of a planned randomized component of this trial. Plans for further development of T-VEC in HNSCC

| Modification | Rationale |
|---|---|
| HSV-1 strain, JS1 | Improves tumour-cell lysis compared with other strains |
| Deletion of ICP34.5 | Provides tumour-selective replication |
| Deletion of ICP47 | Prevents ICP47 from blocking antigen presentation (restores antitumour immune response) |
| Early/increased US11 (as a result of ICP47 deletion) | Increases replication of ICP34.5-deleted HSV-1 in tumour cells |
| Insertion of human GM-CSF (2 copies replacing ICP34.5) | Enhances antitumour immune response |

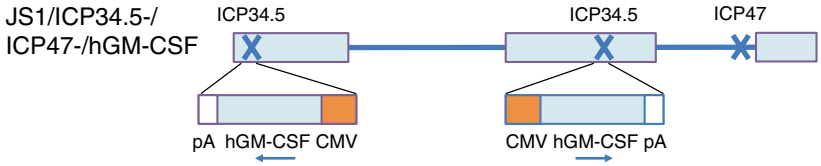


**Fig. 22.4** Biological basis for tumor-selective killing by talimogene laherparepvec (T-VEC). The clinical isolate (JS1) has enhanced potency and is genetically modified to remove both copies of the neurovirulence factor gene (ICP34.5) and the ICP47 gene. This latter modification removes the ability of the virus to prevent antigen presentation (because ICP47 protein blocks the TAP1/2 proteins that mediate antigen transport into the endoplasmic reticulum) and also unleases the activity of the US11 viral promoter earlier in the viral life cycle to promote viral replication. T-VEC expresses human granulocyte-macrophage colony-stimulating factor to recruit and activate dendritic cells in the tumor microenvironment. **Key**: CMV—cytomegalovirus promoter; HSV—herpes simplex virus; ICP—infected cell protein; GM-CSF—human granulocyte-macrophage colony-stimulating factor; pA—polyadenylation signal

were deprioritized in favor of studies focused on melanoma and other histotypes of cancer.

Further studies of HSV-based OV strategies include the development of viruses from the Replimune platform (RP1, RP2 and RP3). RP1 is a replication-competent, enhanced-potency oncolytic HSV-1 that was generated from a new clinical strain of HSV-1 (RH018) [18]. It was modified to express the gibbon ape leukemia virus fusogenic membrane glycoprotein with the R sequence deleted (GALV-GP-R−) to increase oncolysis and promote immunogenic cell death [18]. Similar to T-VEC, RP1 expresses a codon-optimized version of human GM-CSF, which modulates a number of aspects of dendritic cell function [18]. In preclinical *in vitro* and *in vivo* model systems, the expression of GALV-GP-R− improved RP1's oncolytic activity and increased effects in uninjected (anenestic) tumors. The presence of the GALV-GP-R− construct also promotes immunogenic cell death. RP1 has been tested in the phase 1/2 IGNYTE clinical trial (NCT03767348) in combination with nivolumab in patients with skin cancer. The regimen yielded durable responses, with a median duration of response of 13.3 months, including responses in patients with cutaneous melanoma whose disease was not responsive to anti–programmed cell death-1 (PD-1) or anti–PD-1/anti–cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy (6/16; ORR 37.5%). Durable anti-tumor activity was also observed in patients with anti–PD-1–naïve non-melanoma skin cancers (squamous cell, basal

cell, Merkel cell) with a median (range) duration of response of 7.3 (1.9–23.1) months. Consistent increases in both CD8+ tumor-infiltrating lymphocytes and PD-1 ligand (PD-L1) expression were seen where biopsies were available. Based on these results, RP1 is also being tested in combination with the anti–PD-1 antibody cemiplimab versus cemiplimab alone in an ongoing, randomized phase 2 trial (NCT04050436). It is hoped that future studies will expand recruitment to include patients with HNSCC.

The RP1 viral backbone has served as the template for further generations of the RP platform. RP2 and RP3 were designed specifically to counteract the immunosuppressive microenvironment that is found in deposits of cancer in visceral sites, notably the liver. RP2 has been engineered to express an anti–CTLA-4–like antibody with a view to achieving immune priming at the injected tumor site while mitigating against the risk of immune-related adverse events that arise with systemic administration of anti–CTLA-4 therapy [19]. Thus far, in a phase 1 clinical trial (NCT04336241), RP2 has shown good tolerability and has delivered encouraging clinical results in patients with advanced solid cancers, including salivary cancer. In an early study in patients who have failed prior anti–PD-1 therapy, RP2 in combination with nivolumab demonstrated a response rate of four of nine patients with cutaneous melanoma, two of eight patients with uveal melanoma, and one of three patients with HNSCC. RP3 has also been developed as a means of leveraging increased understanding of positive and negative immune signals and their impact on effective immunotherapy. RP3 contains the same genetic modifications as RP2, with the exception that it does not encode GM-CSF and expresses two immune costimulatory pathway–activating ligands, CD40 ligand (CD40L) and 4-1BB ligand (4-1BBL). These features encompass multiple aspects of the innate and adaptive immune response including antigen-presenting cell (APC) activation, T-cell costimulation, and inflammatory cytokine release. RP3 is currently being tested with and without nivolumab in a phase 1 clinical trial in patients with solid tumors (NCT04735978).

## *Genetically-Modified Yersinia Bacterial Therapy*

T3P-Y058-739, abbreviated to T3P, is a genetically-modified, live, attenuated bacterium, *Yersinia enterocolitica*, developed as a treatment for patients with cancer. Naturally occurring *Y. enterocolitica* is an enterobacterium that is acquired by the oral route and is a common cause of gastrointestinal disease. Pigs are the main animal reservoir for pathogenic *Y. enterocolitica* and eating raw or undercooked pork is the main route of transmission to humans. Pathogenic *Y. enterocolitica* infection causes acute febrile diarrhoea, often accompanied by severe abdominal pain that can mimic appendicitis. It may present as enteritis, terminal ileitis, or mesenteric lymphadenitis with diarrhoea. *Y. enterocolitica* usually causes a self-limiting disease but complications can occur with disseminated infection, typically involving the reticulo-endothelial system in the spleen and liver. Bacteraemia and systemic

disease is more likely to occur in older people and in patients with underlying immunosuppressive conditions such as cirrhosis, diabetes or cancer. Iron overload, for example with hemochromatosis, hemoglobinopathies and transfusion dependence, and treatment with the iron-chelating agent desferrioxamine are important predisposing factors for disseminated Yersinia infection.

Yersinia bacteria have the ability to nano-inject surrounding eukaryotic cells with proteins using a bacterial type 3 secretion system (T3SS), which functions as a type of nano-syringe. The T3SS is responsible for the pathogenicity of Yersinia, which uses this system to nano-inject Yersinia outer proteins (Yops) into phagocytes of the innate immune system and thereby disable their key functions. For clinical testing, the following modifications have been made to *Y. enterocolitica* E40 to generate T3P: (i) the natural ampicillin resistance gene (beta-lactamase A) present in *Y. enterocolitica* E40 has been removed. The resulting strain, called *Y. enterocolitica* MRS40 is more sensitive to ampicillin than wild-type Yersinia; (ii) six endogenous effector proteins related to the T3SS (Yops T, H, O, M, P and E) have been removed by mutation, rendering the bacterium less well able to spread and reducing shedding of the agent by infected individuals; (iii) the agent is transgenic for two encoded immunostimulatory, interferonogenic genes which encode the human retinoic acid-inducible gene-1 (RIG-1) caspase recruitment domain (CARD) and cyclic GMP-AMP synthase (c-GAS) proteins.

Thus, T3P is a live attenuated bacterium that has been modified to blunt its pathogenicity and to enable it to nano-inject surrounding eukaryotic cells with two immunostimulatory proteins to trigger the production of type I interferons and other cytokines. The presence of T3P in tumors is anticipated to result in stimulation of a strong immune response against the tumor. Preclinical data for T3P and clinical studies of other live attenuated bacteria in patients with cancer suggest that T3P treatment is likely to be tolerable. The main side effects are expected to be transient pyrexia and coryzal symptoms which, at least in part, will occur as a result of bacterial endotoxin(s). The risk of systemic yersiniosis, and of colonization and/or abscess formation in non-tumor tissue, are expected to be low and manageable with a range of standard antibiotics. Patients with risk factors for these events, notably patients with neutropenia, iron overload or implantable prostheses/devices that cannot be readily removed or changed, will be excluded from the first trial which is currently taking place with direct intratumoral injections of the bacterium in patients with a range of tumor types.

## *Plant Diterpene Ester (Tigilanol tiglate)*

Tigilanol tiglate is a novel, short-chain diterpene ester in early clinical development for direct local treatment of a range of solid tumors [20]. It has a multi-modal action including targeting and activating specific isoforms of protein kinase C (PKC), creating a cascade of intracellular signals which generate host responses against the tumor. Tigilanol tiglate induces a rapid, but highly localized, inflammatory response,

markedly increasing permeability of the tumor vascular endothelium, and causing rapid tumor cell death by oncosis. Together, these effects result in rapid tumor destruction and subsequent sloughing of the treated tumor within 4–14 days and this is usually seen as the development of a deficit or wound. This reaction is often associated with changes in cytokine signalling and gene expression at the tumor deficit site that promote a favorable wound healing response without requiring other interventions. The release of cytokines/chemokines is known to stimulate immune cell recruitment/activity and DAMPS which promote antigen uptake and the development of anti-tumor immunity.

Tigilanol tiglate has recently been registered as a veterinary pharmaceutical treatment for mast cell tumors in dogs by the European Medicines Agency (EMA), the Veterinary Medicines Directorate (VMD) in the UK, the US Food and Drug Administration (FDA) Center for Veterinary Medicines and the Australian Pesticides and Veterinary Medicines Authority (APVMA).

The safety and tolerability of single intratumoral injections of tigilanol tiglate has been investigated in a phase I first-in-human, open-label, dose-escalation study with intratumoral administration in 22 patients and in a repeat-dosing extension study in 9 patients (who had successfully completed the initial dose-escalation protocol and elected to continue in the extension study) [21]. Patients with solid tumors amenable to injection were recruited for the study including squamous cell carcinoma, skin and head and neck cancer (n = 10), melanoma (n = 3), basal cell carcinoma (n = 2) and breast adenocarcinoma (n = 2) with a number of other tumor types being represented by single patients. The intratumoral dose levels of tigilanol tiglate ranged from 0.06 to 3.6 mg/m$^2$, with greatest exposure in a patient who received three doses at 3.6 mg/m$^2$. A maximum tolerated dose (MTD) was not declared.

Ongoing studies in patients with HNSCC (and other head and neck cancer types) and various malignant connective tissue tumors are ongoing in the UK and USA, respectively. These studies include translational biological studies as a means of understanding more about the mechanism of action of tigilanol tiglate.

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# Chapter 23
# Metronomic Chemotherapy and Low-Dose Immunotherapy: An Intriguing Story

**Venkatesh Kapu, Vanita Noronha, Nandini Menon, Minit Shah, Shripad Banavali, and Kumar Prabhash**

## Introduction

Current treatment protocols are not curative for some cancers, despite significant advancements in cancer research. Furthermore, adverse drug reactions from traditional chemotherapy, despite novel drug delivery methods, limit drug dosages and impair the effectiveness of anti-neoplastic medications. To enhance the treatment of cancer, it is therefore crucial to investigate novel therapeutic targets and approaches.

In 2000, Kerbel and Hanahan introduced the treatment known as metronomic chemotherapy (MCT) [1, 2]. The basis for this therapeutic approach was established by the research groups of J. Folkman and R. Kerbel. In contrast to conventional chemotherapy regimens, this novel and effective treatment approach for solid tumors and blood malignancies entails the continuous administration of low-dose cytotoxic medications. While MCT is utilizing the drugs at low doses that are significantly below the maximum tolerable dose (MTD) without long intervals between doses, conventional therapy is based on the administration of maximum doses of these drugs at specific intervals, as determined by normal tissue recovery [3]. The main characteristics of MCT are: frequent, uninterrupted, dose-dense chemotherapy administration; use of a biologically optimized dose instead of MTD; low incidence of treatment-related side effects; inclination toward oral medications; and potential for delayed resistance development [4]. It may increase the therapeutic index of drugs since it offers a good balance between the treatment's effectiveness and side effects. Additionally, it permits longer treatment, which most likely improves survival. Oral medications are a better option for patients with MCT because it necessitates consistent drug delivery.

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## Mechanism of Action of Metronomic Chemotherapy

Firstly, solid tumor cells typically belong to a heterogeneous group with varying angiogenic potential and cell kinetics. Secondly, due to inherent genomic instability, tumor cells may develop resistance to chemotherapy. Finally, since metastases are frequently the cause of tumor relapse and tumor-related death, conventional chemotherapy typically has less effect against metastases than on the primary tumor [5]. MCT is a multi-target therapy because it has impact on cancer cells and their surroundings both directly and indirectly. It could even induce tumor dormancy, suppress tumor angiogenesis, and strengthen the immune system's defence against tumors [6].

By inducing hypoxia and nutrient starvation, drugs that target angiogenic pathways cause damage to tumor cells. Anti–vascular endothelial growth factor (Anti-VEGF) monoclonal antibodies (Moabs) are another class of therapy that shares a similar mode of action. However, they have a wide range of characteristics. Tumoral endothelial cells (TEC) may be a better target for overcoming drug resistance than VEGF, as the antiangiogenic drug directly targets VEGF's action, while oral MCT (OMCT) inhibits the cells involved in angiogenic mechanisms. In addition to its notable effects on endothelial cells, MCT also encourages the induction of thrombospondin-1, an antiangiogenic protein that inhibits angiogenic hypoxia inducible factor 1 subunit alpha (HIF-1α) and lowers levels of circulating VEGF [5].

Cancer is to a large extent immune-related, and one of the main reasons that cancer grows is its ability to evade immunosurveillance. Adoptive immunity as well as innate immunity are crucial for the growth arrest of cancer cells. However, currently the most widely accepted theory of action centers around regulatory T cells (Tregs). Tregs are defined as CD4+, FOXP3+, CD25+ lymphocytes that contain the cytotoxic T lymphocyte associated antigen 4 and glucocorticoid-induced tumor necrosis factor (TNF) receptor. These lymphocytes block the anti-tumor immune response by suppressing both tumor-specific and nonspecific natural killer (NK) cells. Numerous malignancies have been demonstrated to have elevated Tregs. Ghiringhelli et al.'s research [7] demonstrated the efficacy of low doses cyclophosphamide (CTX) in suppressing the immune effects of Tregs in a variety of human malignancies. They added that the function of NK cells and immune-specific cytotoxic T cells could be restored by using lower dosages of CTX. Because cytotoxic drugs kill immune cells, high-dose chemotherapy can compromise the immune system's ability to fight cancer cells that are resistant to treatment. On the other hand, regular low-dose chemotherapy can promote the maturation of antigen-presenting cells (APCs), increase dendritic cell activity, destroy immune-suppressive Tregs, and, most importantly improve the activation and functionality of cytotoxic NK and CD8+ T cells. Moreover, myeloid-derived suppressor cells (MDSCs) are affected by MCT drugs also, which lessens the suppression of adaptive immune responses and increases anti-tumor activity [8].

Three main mechanisms are involved in the initiation of tumor dormancy by MCT, i.e., immune surveillance, angiogenesis suppression, and programmed cell death of malignant cells [9].

## Metronomic Chemotherapy in Head and Neck Cancer Patients

Celecoxib and methotrexate are the two main medications recommended for OMCT in numerous publications. The primary mechanism of action of celecoxib is based on its inhibition of cyclooxygenase-2 (COX-2). The majority of malignant tumors in the head and neck exhibit COX-2 overexpression. There is additional evidence to support the theory that COX-2 overexpression causes tumorigenesis [10]. Moreover, lymph node metastases in patients with head and neck cancer are associated with COX-2 overexpression. The second important medication used in OMCT is methotrexate (MTX). It is folic acid's 4-amino 10-methyl analogue. Dihydrofolate reductase, an essential enzyme for maintaining intracellular folates in reduced form, is bound to it and inhibited by it. This reduced state is necessary for the synthesis of nucleic acid, which breaks deoxyribonucleic acid (DNA) strands when deficient. Additionally, it inhibits the synthesis of DNA by introducing abnormal nucleotides into DNA and directly inhibiting enzymes that depend on folate.

### *Triple Metronomic Chemotherapy*

Chemotherapy administered metronomically has been proposed as away to overcome treatment resistance. In the first line setting for head and neck cancer, MCT, which consists of oral weekly MTX and celecoxib, has demonstrated promising activity. However, in patients who failed early or were not sensitive to platinum, this combination produced disappointing outcomes [11]. The overexpression of ATP-binding cassette super-family G member 2 (ABCG2), a multidrug transporter that facilitates the quick removal of MTX and 7-hydroxymethotrexate from cells, is one of the mechanisms of MTX resistance. Of head and neck cancers, 50–65% overexpress the ABCG2 transporter. Erlotinib has demonstrated encouraging efficacy in patients resistant to cisplatin and metronomic MTX. It reverses the ABCG2-mediated drug efflux function [12]. Angiogenesis may play a role in the development of erlotinib resistance, while the main mechanism of action of MCT is anti-angiogenesis.

A thorough analysis of numerous studies that have recently been published regarding the use of OMCT in head and neck cancers revealed that OMCT is particularly helpful in the palliative, recurrent and metastatic setting. There are some limited data supporting its use in neoadjuvant settings and as maintenance in adjuvant therapy.

## *Oral Metronomic Chemotherapy in the Palliative Setting*

Numerous pilot studies and trials have been conducted in accordance with the hypothesis and mechanism of action that have been proposed above. The results of these trials indicated that there were several factors influencing the OMCT outcome. The time to failure is one of the most important factors; patients who fail within 6 months have a worse overall survival rate than those who fail beyond 6 months. Essentially, patients with platinum-refractory disease, who typically perform worse, make up this subgroup of patients who fail within 6 months. The EXTREME trial [13], showing a better progression-free survival (PFS) with the addition of cetuximab to platinum-based chemotherapy versus platinum-based chemotherapy alone (5.6 vs 3.3 months, respectively) did not include patients with platinum-refractory disease—those who failed within 6 months. In contrast, a study by Patil et al. [14], a randomized phase II trial comparing OMCT [daily celecoxib (200 mg twice daily) and weekly methotrexate (15 mg/m$^2$)] to intravenous single agent cisplatin (IP) (75 mg/m$^2$) given 3 weekly, also included patients who failed within 6 months. This prospective study showed a PFS of only 66 days in the chemotherapy-based arm. The OMCT arm outperformed the chemotherapy arm interms of median PFS (101 days vs 66 days, $p = 0.014$), median overall survival (OS: 249 days vs 152 days, $p = 0.02$), less grade 3/4 adverse events (18.9% vs 31.4%, $p = 0.14$) and a better safety profile and quality of life. These improved outcomes in platinum-refractory cases may be explained by OMCT's antiangiogenesis effects and actions on the endothelial cells in the tumor's supplying vasculature. Given that endothelial cells in the vasculature are essentially genotypically stable, somatic mutations in the tumor that cause platinum resistance should not have an impact on them.

The importance of sites and subsites for OS and PFS are other significant factors that have been described in a number of studies. As such, it has been reported that pharyngeal and laryngeal primaries perform better in terms of OS and PFS than advanced or recurrent oral cavity primaries. In oral cavity primaries, surgery is generally thought to yield better results than chemotherapy; however, in practice, in our population, only approximately 16% of these patients present at a stage that can be surgically treated. While oral cavity primaries make up the majority of trials and studies included in our review, which represents an Indian subset of the population, only about 21% of patients in the study arm and 19% in the control arm were part of the mixed subset of patients in the EXTREME trial reported by Vermorken et al. [15] who found that patients treated in the cetuximab arm of the study performed better in terms of OS than those who were treated in the chemotherapy only arm of the study. Therefore, the performance of the OMCT medication regimen in comparison to the established regimen is quite satisfactory when compared to other studies. Further investigation into the relationship between HPV and other variables, as well as the variation in response rates between the oral cavity and other head and neck primaries (pharyngeal and laryngeal), is necessary.

Quality of life (QOL) and related side effects are a crucial factor that shifts the scales in favour of the OMCT. It has been observed that the quality of life (QOL) of patients undergoing OMCT is significantly improved from baseline values at intervals of 3–6 months, with respect to functional scales such as pain, difficult swallowing, dry mouth, mouth opening, sticky saliva, social eating, and social contact.

The majority of the trials mentioned above used medications for double metronomic therapy; there aren't many studies on triple metronomic chemotherapy. In order to determine the effectiveness of this three-drug regimen in treating platinum-resistant or early-failure squamous cell carcinoma of the oral cavity, Patil et al. [11] conducted a phase I/II study combining MTX when given in addition to erlotinib and celecoxib. The 3-months PFS in that study was 71.1% (95% CI, 60.5–79.3%), its 6-month OS 61.2% (95% CI, 49.2–67.8%), and the overall response rate (ORR) was 42.9% (95% CI, 33.2–53.1%; n = 39). A phase 3 open-label study executed by Kapoor et al. [23] in 214 patients with advanced HNSCC (previously treated with platinum-based chemotherapy, and 65.9% had oral cavity cancer) were randomised 1:1 to receive triple OMCT (Arm A) versus physician's choice (Arm B). Results revealed a median OS of 9 months in Arm A and 5 months in Arm B (HR, 0.63; 95% CI, 0.47–0.83; p = 0.00011). Median PFS was 4 months in Arm A and 2 months in Arm B (HR, 0.67; 95% CI, 0.52–0.87; p < 0.0001). Table 23.1 summarises the results of studies in this setting.

Kothari et al. [24] conducted a phase 3 multicentric study (16 sites) in patients with locally advanced head and neck squamous cell carcinomas which had recurred and were metastatic and either platinum-refractory or planned for second-line chemotherapy. Triple MCT as second-line therapy versus physician's choice showed a median OS of 181 days (95%CI 142.7–219.2) versus 123 days (95%CI 94–152), 6-month OS of 52.9% (95%CI 36.9–65.1) versus 14.8% (95%CI 6.4–26.4) and a median PFS of 120 days (95%CI 89.2–150.8) versus 70 days (95% CI 58.2–81.8) (p = 0.000), respectively. With these results, the authors concluded that triple MCT as second-line therapy had an OS and PFS advantage over NCCN-recommended physician's choice therapy.

## *Oral Metronomic Chemotherapy in Adjuvant and Neoadjuvant Settings*

The best treatment for advanced oral cavity cancer is complete surgical resection with R0 margins. However, because of overall poor outcome in advanced disease cases, several studies have suggested further exploring the role of chemotherapeutic agents in resectableoral cancers. Notable research in this area includes studies by Licitra et al. [25] and Zhong et al. [26], both showing no benefit of neoadjuvant (induction) chemotherapy in terms of OS or disease-specific survival (DSS) benefit.

The effectiveness and safety of triple OMCT (MTX, celecoxib, and erlotinib) and paclitaxel-carboplatin were evaluated by Kashap et al. [27] in 14 patients with

**Table 23.1** OMCT in head and neck cancer: summary of studies

| Study | Disease setting/study population | Type of study | Number of patients | Treatment regimen | Outcomes | Results |
|---|---|---|---|---|---|---|
| Patil VM et al. (2015) [14] | Metastatic, relapsed or inoperable HNSCC | Phase II | 110 | Celecoxib 200 mg twice daily + methotrexate 15 mg/m$^2$ weekly ($n$ = 57) versus cisplatinum75 mg/m$^2$, 3 weekly ($n$ = 53) | OS, PFS | PFS 101 versus 66 days ($p$ = 0.014); OS 249 versus 152 days ($p$ = 0.02) |
| Geetha et al. (2016) [10] | Recurrent/metastatic | Single arm prospective observational study | 15 | Methotrexate 15 mg/m$^2$/week, oral celecoxib 200 mg twice daily and erlotinib 150 mg once daily | PFS | PFS:148 days |
| Kalaichelvi K (2016) [10] | Advanced/recurrent head and neck squamous cell carcinoma (HNSCC) | Retrospective | 30 | Weekly oral methotrexate 5 mg twice daily for 2 days/week and oral celecoxib 200 mg twice daily | CBR, QOL | Pain control: 80%, QOL improved |
| Patil VM et al. (2020) [16] | Relapsed/recurrent or newly diagnosed | Phase III | 422 | Weekly methotrexate 15 mg/m$^2$ + celecoxib 200 mg twice daily (n = 213) versus 75 mg/m$^2$ IV cisplatin once every 3 weeks (n = 209) | OS | OS 7.5 versus 6.1 months (p = 0.026) OMCT was noninferior to IV cisplatinum |
| Patil VM et al. (2015) [17] | Oral squamous cell carcinoma patients with early failure and/or platinum-resistant disease | Retrospective | 100 | Weekly methotrexate 15 mg/m$^2$ + celecoxib 200 mg twice daily | OS, 6 months survival | OS: 110 days, 6 months survival: 26.4%. OMCT failed to meet its prespecified efficacy limit |
| Shilpa Kandipalli et al. (2018) [18] | Recurrent, residual and metastatic head & neck cancers | Prospective observational Study | 47 | Methotrexate 2.5 mg twice weekly Capecitabine: 500 mg twice daily ($n$ = 47) | Quality of life | Improved QOL |

| | | | | | | |
|---|---|---|---|---|---|---|
| Patil VM et al. (2017) [19] | Unselected cohort of head and neck cancer | Retrospective | 340 | Methotrexate 15 mg/$m^2$ twice weekly + celecoxib 200 mg twice daily | OS | OS: 155 days, Promising results in selected group of patients |
| Patil VM et al. (2012) [20] | Metastatic, recurrent and locally advanced oral cavity cancers which were not amenable to local treatments | Retrospective | 18 | Weekly methotrexate 15 mg/$m^2$ + celecoxib 200 mg twice daily | Toxicity profile and efficacy | Toxicity: minimal, CBR: 44.48%, Symptom control: 66.7%, PFS: 5 months, OS: 3.05 months |
| Kumar KSS et al. (2019) [21] | Advanced/recurrent HNSCC | Phase II study | 50 | Methotrexate 15 mg twice daily for 2 days/week + celecoxib 200 mg twice daily | Overall response rate | DCR: 76% (2 months) and CBR:64% (6 months) |
| Noronha V and Patil VM (2017) [22] | Metastatic/recurrent head and neck squamous cell cancer | Match pair analysis | 120 | Weekly Paclitaxel 80 mg/$m^2$ + cetuximab (60) versus MCT (60) | OS | OS: 191 days in MCT versus 256 days in cetuximab cohort |

technically unresectableoral squamous cell carcinoma as neoadjuvant chemotherapy (NACT). The buccal mucosa and oral tongue were the primary tumor sites in 12 (86%) and 2 (14%), respectively. Nine cases (65%) became resectable following NACT. The median PFS was 11.4 months (95% CI, 7.9–15 months), the median OS had not been reached at time of reporting, and the OS at 15 months was 63.5% (95% CI, 37.8–89.2%). Pathak et al. [28], using a similar approach, identified 72 patients for NACT who had borderline resectable or unresectable disease. The ORR was 61.1%, 34 of 40 (85%) borderline resectable patients underwent surgery and overall estimated PFS and OS were 18.5 (95%CI = 14.4–22.7) months and 18.05 (95%CI = 14.2–21.8) months, respectively.

Pai et al. compared two categories of patients: those who underwent preoperative metronomic chemotherapy, followed by surgery and adjuvant therapy with maintenance OMCT, and those who underwent direct surgery followed by adjuvant therapy. Of 32 Patients analysed disease-free survival (DFS) rates after 2 years were 71.6% in the control group and 86.5 in the OMCT group. Additionally, the 2-year DFS for patients who received adjuvant OMCT for at least 3 months was 94.6%. Even though the short-term outcomes of several studies by various authors are similarly encouraging, we must wait for long term results [29].

In a phase 2 randomized study, Patil et al. [30] evaluated the efficacy of adjuvant MCT in patients with recurrent head and neck cancers after salvage surgery who were not eligible for re-irradiation. The patients in the OMCT arm had a 1-year and 2-year DFS of 57.4% (95% CI, 42.8–69.5) and 37.6% (95% CI, 24.1–51) respectively, while those in the observation arm had 62.3% (95% CI, 47.8–73.8) and 54.2% (95% CI, 39.8–66.5), respectively (HR for progression, 1.45; 95% CI, 0.87–2.47; p = 0.15). The 1- and 2-year OS in the OMCT arm were 78.7% (95% CI, 64.9–87.6) and 48% (95% CI, 34.1–62), respectively, while the observation arm had 79.2% (95% CI, 65.7–87.9) and 65.5% rates (95% CI, 50.9–76.7), respectively (HR for death, 1.7, 95% CI, 0.94–3.08; p = 0.08). These results suggested that the adjuvant 6-month metronomic schedule was ineffective in improving outcomes in recurrent head and neck cancers following salvage surgery who are not eligible for re-radiation.

## *Basis for Low Dose Immunotherapy*

Immune checkpoint inhibitors (ICIs) have revolutionized the way many cancer types are treated, but they have also brought about a number of new immune-related adverse events (irAEs) that need to be managed and an additional exponential rise in direct and indirect costs. Guidelines from the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) recommend nivolumab or pembrolizumab in the platinum-refractory setting [31, 32]

and KEYNOTE-048 (pembrolizumab plus a platinum and 5-fluorouracil) or the EXTREME regimen (platinum-5-fluorouracil and cetuximab) in the first-line setting [13, 33]. Just 1–3% of patients with recurrent or metastatic head and neck squamous cell carcinoma can access programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) immune checkpoint inhibitors in low- and middle-income countries [34, 35].

Long-term pharmacokinetic (PK) and pharmacodynamic (PD) data have provided support to the idea that immunotherapy may function just as well—possibly even better in terms of immune-related toxicity profile—at dosages much lower than those that are currently approved.

When used in clinical trials, receptor occupancy assays may give insight on the PK/PD relationships between immune checkpoint receptor-targeting pharmaceuticals. PD-1, which is highly expressed on the surface of circulating T cells and ready for additional examination by peripheral blood sampling. PD-1 occupancy following nivolumab administration appears to be dose-independent, according to the first in-human, phase 1 dose-escalation study [36], with a mean peak occupancy of 85% right after infusion and a mean plateau occupancy of 72%. Furthermore, even in cases where serum nivolumab levels were below the lower limit of quantification, the study demonstrated a long-lasting PD-1 occupancy. Another study found that all dose cohorts (0.3, 2, and 10 mg/kg) had similar receptor occupancy profiles, and that PD-1 occupancy of ≥90% was reached within an hour of nivolumab administration and stayed close to this level for the duration of the treatment cycle [37].

In a 2012 phase I trial, 0.1 mg/kg of nivolumab (or roughly 3% of 3 mg/kg) administered every other week demonstrated comparable levels of activity and receptor saturability to higher dosages [38].

Pembrolizumab (P), another anti-PD-1 monoclonal antibody that is currently widely used in cancer treatments, was found to operate on the same principle [39].

## *Rationale for Combining Metronomic Chemotherapy and Immunotherapy*

Originally intended to overcome drug resistance by changing the therapeutic target from tumor cells to tumor endothelial cells, MCT is defined as the frequent administration of chemotherapeutic agents at a non-toxic dose without prolonged rest periods. Additionally, MCT has anti-tumor effects on tumor cells and the immune system (immunomodulation). Boosting host anti-tumor immunity is the aim of immunotherapy. Combining immunotherapy with chemotherapeutic drugs can stimulate the production of tumor antigen-specific T lymphocytes in the host, which

can enhance the anti-tumor effects in a synergistic manner. Through at least three immune modulation mechanisms, MCT can overcome the host's immunosuppressive state: firstly, by improving dendritic cells' ability to present antigens; secondly, by encouraging the development of protective T-cell responses; and thirdly, by reducing immunosuppression in the tumor bed. These effects have the potential to enhance the anti-tumor effects of immunotherapies when coupled with them. Therefore, a sensible approach to enhancing immune surveillance in cancer treatment can involve the combination of metronomic therapy and immunotherapy.

## Clinical Data for Metronomic Chemotherapy and Immunotherapy in Head and Neck Cancers

In a randomized phase III superiority study, Patil et al. [40] showed that the addition of low-dose nivolumab to MCT improved OS and represents an alternative standard of care for patients unable to access full-dose checkpoint inhibitors. The patients were assigned 1:1 to MCT consisting of oral methotrexate 9 mg/m$^2$ once a week, celecoxib 200 mg twice daily, and erlotinib 150 mg once daily, or MCT plus intravenous nivolumab (MCT-I) 20 mg flat dose once every 3 weeks, while the full-dose administration is 200 mg every 2 weeks. A total of 112 patients were randomly allocated, with 75 placed in the MCT arm and 76 in the MCT-I arm. A 1-year OS improvement was observed with the addition of low-dose nivolumab: 16.3% (95% CI, 8.0–27.4) to 43.4% (95% CI, 30.8–55.3; HR, 0.545; 95% CI, 0.362–0.820; p = 0.0036). As for the MCT and MCT-I arms, the median OS was 6.7 months (95% CI, 5.8–8.1) and 10.1 months (95% CI, 7.4–12.6) (p = 0.0052), respectively. There is interest in exploring the combination of immunotherapy and metronomic chemotherapy in head and neck cancer. Table 23.2 provides the ongoing trials for this combination.

**Table 23.2** Ongoing clinical trials with metronomic chemotherapy and immunotherapy

| OMCT | ICI | Study title | Status | Phase | Clinical Trial n° |
|---|---|---|---|---|---|
| Cyclophosphamide | Nivolumab/ Ipilimumab | Autologous dendritic cells and metronomic cyclophosphamide in combination with checkpoint blockade for relapsed high-grade gliomas in children and adolescents | Recruiting | I | NCT03879512 |
| Vinblastine Cyclophosphamide Capecitabine | Nivolumab | Nivolumab in combination with metronomic chemotherapy in pediatrics refractory/ relapsing solid tumors | Recruiting | I and II | NCT03585465 |
| Gemcitabine Doxorubicin Docetaxel | Nivolumab | GALLANT: Metronomic gemcitabine, doxorubicin, docetaxel, and nivolumab for Advanced Sarcoma | Recruiting | II | NCT04535713 |
| Temozolomide | Nivolumab | Temozolomide + nivolumab in MGMT Methylated Oesophagogastric Cancer (ELEVATE) | Recruiting | II | NCT04984733 |
| Temozolomide | Nivolumab + ipilimumab | A longitudinal assessment of tumor evolution in patients with brain cancer. | Recruiting | I | NCT03425292 |
| Cyclophosphamide | Pembrolizumab | Phase 2 study of an immune therapy, DPX-survival with low dose cyclophosphamide administered with pembrolizumab in patients with persistent or recurrent/refractory diffuse large B-cell lymphoma (DLBCL) | Active, not recruiting | II | NCT03349450 |

(continued)

**Table 23.2** (continued)

| OMCT | ICI | Study title | Status | Phase | Clinical Trial n° |
|---|---|---|---|---|---|
| Decitabine | PD-1 antibody (SHR-1210) | Combined chemotherapy and PD-1 antibody (SHR-1210) with or without low dose decitabine priming for relapsed or refractory primary mediastinal large B-cell lymphoma (rrPMBCL): Two stage, phase I/II trial | Unknown | I and II | NCT03346642 |
| Gemcitabine | Nivolumab | Low dose gemcitabine combined with nivolumab for second-line and above-line treatment of non-small cell lung cancer metastatic | Not yet recruiting | IV | NCT04331626 |
| Cyclophosphamide | Pembrolizumab | CHEMOIMMUNE study: Evaluation of pembrolizumab in lymphopenic metastatic breast cancer patients treated with metronomic cyclophosphamide (safety run-in phase) | Completed | II | EudraCT n. 2016–002736-33 |
| Cyclophosphamide | Avelumab | CONFRONT Phase I–II Trial: Multimodality Immunotherapy with avelumab, Short-Course Radiotherapy, and cyclophosphamide in Patients with Relapsed/Metastatic Head and Neck Cancer | Ongoing | I and II | EudraCT n. 2017-000353-39 |

## Conclusions

Researchers are attempting to find a single cure that will work for all types of cancer. Effectively combining such treatments is crucial for the future of cancer therapy, as evidenced by every new development. Immunotherapy is the next step in cancer treatment and may be more effective against tumors when combined with other treatments that have the capacity to modulate the immune system. In conclusion, immune checkpoint inhibitors combined with MCT may offer a way to target cells that are resistant to therapy without causing unacceptable toxicity. This could lead to increased treatment adherence, better long-term results for cancers that are challenging to treat, and an improvement in the quality of life for patients.

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# Chapter 24
# Metastatic Head and Neck Cancer: What Is the Optimal Local Treatment?

**Petr Szturz and Jan B. Vermorken**

## Introduction

Typically affecting the lungs, bones, and liver, distant metastases of squamous cell carcinoma of the head and neck (SCCHN) occur in several distinct clinical scenarios encompassing newly diagnosed SCCHN with synchronous distant metastases (Type 1), locoregionally controlled SCCHN with metachronous distant metastases (Type 2), and locoregionally uncontrolled SCCHN with synchronous or metachronous distant metastases (Type 3) (Fig. 24.1). Different stages of local or locoregional disease pertain to the first category, ranging from T1N1 to T4N3 according to the tumour-node-metastasis (TNM) staging system. The second category includes both newly diagnosed primary tumours that have been treated and controlled but have recurred at distant sites, as well as locoregional recurrences that have been treated and controlled but have presented with a new recurrence at distant sites. Finally, the third category also encompasses newly diagnosed and recurrent tumors that, unlike the second category, are characterized by progression and may present with either synchronous or metachronous distant metastases. While synchronous metastases develop concurrently with the index primary cancer, there is a lag of at least 3 months between the diagnosis of the primary tumour and the appearance of metachronous metastases. Newly diagnosed and recurrent SCCHN have different biologies, and distinguishing these two entities has substantial therapeutic consequences due to differences in the available anticancer armamentarium and the amount of information we have about the disease kinetics in individual patients.

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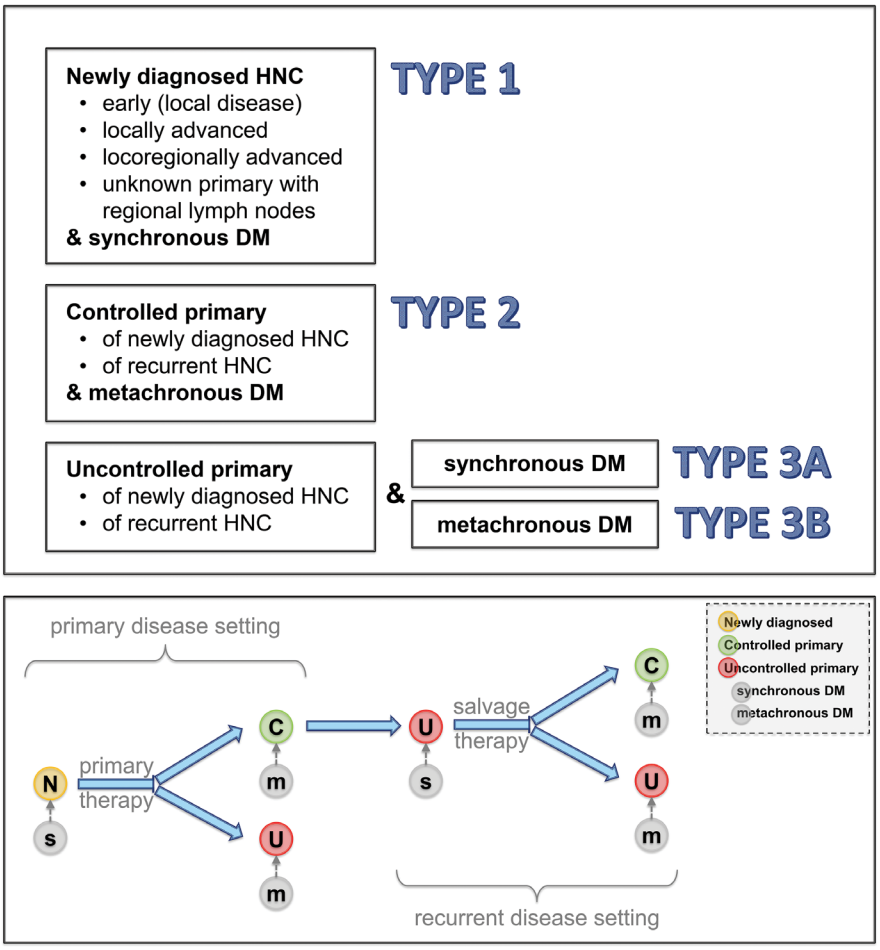


**Fig. 24.1** Typology of distant metastases (DM) in head and neck cancer (HNC) with corresponding stages according to Tumour-Node-Metastasis (TNM) classification. In Type 2, a controlled primary tumour refers to a complete or partial response or stability at the locoregional site. Type 3A involves newly diagnosed or locally and/or regionally recurrent HNC with synchronous DM. In Type 3B, newly diagnosed or recurrent HNC progresses after primary or salvage treatment, respectively, and is accompanied with metachronous DM. The lower panel illustrates the disease course, starting with a newly diagnosed HNC on the left, which can either be controlled or, less frequently, remain uncontrolled following primary therapy. Controlled disease may recur as uncontrolled disease and can either become controlled again after successful salvage therapy or remain uncontrolled despite such treatment. These disease states can be associated with DM, which may present as synchronous if detected within the first three months after the diagnosis of a new HNC case or a recurrence, or as metachronous in all other cases

Owing to the follow-up period prior to the diagnosis of a recurrence, a series of imaging modalities may be available, allowing for an estimation of the disease pace and, subsequently, disease aggressiveness.

In this chapter, we will examine the current standard of care portfolio and use it as a springboard to innovative approaches in the management of metastatic disease.

## Current Therapeutic Concepts

Notwithstanding reimbursement policies, drug availability issues, and individual patient contraindications, a combination of immunotherapy and chemotherapy represents the most universal first-line systemic treatment for the clinical scenarios described in Fig. 24.1. The results of the Keynote-048 trial indeed provide sufficient evidence supporting this notion [1]. In addition, insights from the study subgroup analyses, supported by real-world evidence, have brought further granularity to decision-making [2]. The combined positive score (CPS) emerged as a usable predictive factor, the first of its kind in SCCHN, dichotomizing first-line systemic treatment into biochemotherapy with cetuximab/platinum/5-fluorouracil, appropriate for tumours with no expression of programmed cell death ligand-1 (PD-L1) manifesting as a CPS of 0, and immunochemotherapy, which employs the same regimen except for replacing cetuximab with the immune checkpoint inhibitor and monoclonal antibody against PD-1, pembrolizumab. Immunochemotherapy is thus better suited for PD-L1 positive cases with a CPS of 1 or more. While chemotherapy alone is usually not given, immunotherapy using pembrolizumab alone can furnish significant efficacy in a subgroup of higher PD-L1 expressors (CPS $\geq$20), and this with a remarkably low toxicity burden. However, there are still some downsides to immunotherapy, including a low rate of objective response, the risk of hyperprogression, immune-related adverse events, and high cost. As an alternative to the standard biochemotherapy regimen EXTREME, fit patients may be candidates for TPEx, which replaces 5-fluorouracil with docetaxel [3].

The decision-making process is a complex procedure that takes into account the global treatment strategy as well as individual patient- and disease-related factors. The former is reflected in treatment sequencing planning, which was addressed in the previous edition of *Critical Issues in Head and Neck Oncology* [4]. The starting point for this planning involves, when applicable, a curative approach for locoregionally advanced disease and continues with subsequent treatment lines after the failure of first-line bio- or immuno-chemotherapy. The latter aspects, comprising patient- and disease-related factors, were also discussed in the previous edition of the book. Here, we present an algorithm for first-line systemic treatment, acknowledging the complexity of decision-making on the one hand side and practicality on the other (Fig. 24.2). Platinum eligibility and patient health status are probably the first considerations. Patients in good health with no contraindications to platinum, including a long disease-free interval after previous platinum administration, can usually benefit from all available treatment schedules. In those with contraindications to cisplatin, carboplatin can be considered, but if the patient presents with a recurrence within 6 months after previous platinum administered in the curative setting, then such treatment should be abandoned, and single-agent immunotherapy or a taxane ± cetuximab should be opted for. Patients with poor performance status were not included in the registration clinical trials, leading to limited supporting evidence for decision-making. These patients are usually offered less intensive treatment with fewer agents, palliative radiotherapy or surgery, or supportive care alone. Importantly, advanced chronological age is not a contraindication *per se* for full-dose standard-of-care treatment, which can therefore be administered to elderly

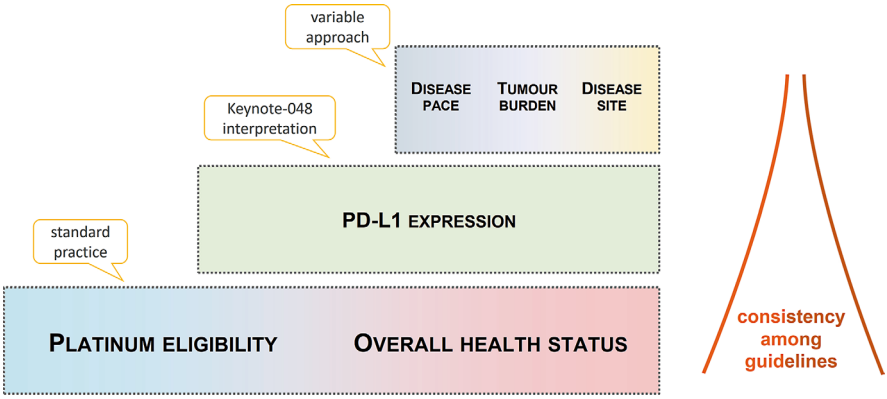


**Fig. 24.2** A stepwise decision-making algorithm for systemic treatment in recurrent and/or metastatic squamous cell carcinoma of the head and neck. Consistency among different guidelines decreases as the guidelines address factors in higher steps. **Abbreviations**: PD-L1, programmed cell death ligand-1

patients who are deemed fit according to geriatric assessment. This approach has been adopted in the majority of clinical practice recommendations. However, in the following steps, some guidelines differ.

As mentioned above, according to Keynote-048, PD-L1 expression should guide us towards biochemotherapy in CPS-negative cases and to immunochemotherapy in the remaining cases, with a CPS of 20 often regarded as the lowest value to allow single-agent pembrolizumab as well. In the final step, disease kinetics, tumour burden, and disease site should all be considered. Disease pace is often equated with disease aggressivity and correlated with treatment intensity, albeit with little support in the scientific literature. Increased tumour burden may steer us away from immunotherapy, particularly when given as monotherapy [5]. Finally, we have started to recognize potential differences in approaching locoregional recurrence versus distant metastases, realizing that in comparison with chemotherapy, immunotherapy is associated with lower response rates but may be more active in cases with distant metastases [2]. The concept of systemic treatment is valid for all types of distant metastases, as depicted in Fig. 24.1.

## New Therapeutic Concepts

Innovation is how progress in oncology has been achieved, uniting researchers, physicians, and patients around a common objective. Innovation has two principal components, invention and improvement. Both can be classified according to their pertinence to either diagnostic or therapeutic procedures, resulting into four categories with different content, as shown in Fig. 24.3. The development of these four fields of interest have been shaped by preventive measures, artificial intelligence,

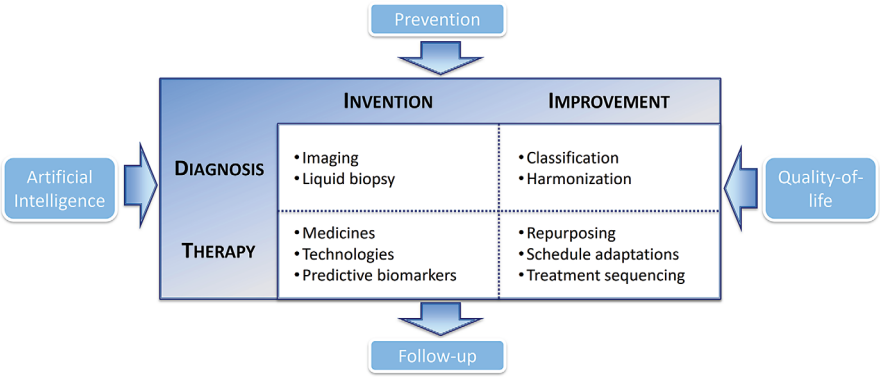


**Fig. 24.3** Four main fields of innovation in medicine with examples pertaining to head and neck oncology

quality of life aspects, and the need to optimize patient follow-up, which should represent the longest part of patient's journey. Each of these areas is relevant to our current topic about local therapy in metastatic disease. In particular, we will focus on the repurposing of local therapy, which in head and neck cancer has been the mainstay for the non-metastatic locoregionally advanced setting. However, other innovations also play a role including improved classification to streamline decision-making, advances in imaging for enhanced detection of distant metastases, liquid biopsy to aid diagnosis in ambiguous cases and hopefully estimate tumour burden in the future, and last but not least, as is always the case, reliable predictive biomarkers, which are nevertheless still regrettably almost absent.

## Oligometastatic Disease

This topic was already addressed in the previous edition of *Critical Issues in Head and Neck Oncology* [6]. Surgery and radiotherapy of oligometastases are the most common examples of local treatment in metastatic disease. Two complementary objectives have been pursued. The first is to achieve substantial tumour debulking to prolong survival, as subsequent progression is inevitable but can be delayed. This has been possible in patients with a small number of distant lesions, usually five or fewer, corresponding to the so-called oligometastases, although the positive impact of tumour burden reduction has also been shown in more extensive dissemination, albeit in a different disease entity (colorectal adenocarcinoma) [7]. Hence, the designation oligometastases embodies a more pragmatic than biological approach, allowing for a rapid identification of cases suitable for local eradication of these metastases using most commonly a surgical pathway, radiotherapy (particularly stereotactic body radiotherapy), and other modalities, including radiofrequency ablation or cryoablation. However, such a definition does not filter out cases with

unrecognized microscopic dissemination, which make up the majority of oligometastatic phenotypes [8]. Consequently, the local approach has often been combined with systemic treatment to maximize survival outcomes.

The latter objective is to offer curative treatment to select patients employing solely local modalities. This can be accomplished if only patients without unrecognized microscopic dissemination are subjected to curative-intent resection or destruction of all visible metastases, the number of which should ideally remain low, which also facilitates ablation. Recently, we have introduced the term "argometastases" as a synonym for true oligometastases. In this context, all visible metastatic lesions represent the only distant sites of malignant dissemination (Table 24.1). In Greek, *argos* means slow, idle, or lazy and refers to the hallmark of argometastases which is the slow speed of tumour cell shedding and proliferation. Ideally, such an entity should be defined and diagnosed using biological criteria, including genetic, epigenetic, and immune determinants. However, our current knowledge is limited in this respect, and we must rely on clinical characteristics. We have divided these traits into three tumour-agnostic categories, comprising a low probability of occult metastases, low tumour growth rate, and low tumour burden. A low probability of occult metastases is based on the findings of a metachronous presentation with a long disease-free interval following previous anticancer treatment, controlled tumour at the primary site, absence of suspicious micronodules of unknown origin (typically in the lungs), absence of regional lymph node involvement at initial diagnosis, favourable distant organ site involvement (e.g., the lungs or liver rather than the peritoneum), the possibility of lowering the detection threshold by auxiliary imaging and laboratory methods (e.g., using specific novel tracers for positron emission tomography or circulating tumour DNA), and a susceptible tumour origin involving not only SCCHN but also other cancers, typically colorectal adenocarcinoma. A low tumour growth rate can be assessed using a series of radiologic follow-up exams. Finally, a low tumour burden aligns partially with a pragmatic approach, necessitating safe and complete local ablation [9]. As mentioned above, the prevalence of argometastases is lower than that of traditional oligometastases. While about half of all metastatic cancers across various tumour types can be radiologically classified as oligometastases, only one fifth of these represent argometastases, i.e., true oligometastatic disease, corresponding thus to about 10% of all metastatic cancer cases (Fig. 24.4) [8, 10].

**Table 24.1** Three types of distant metastatic spread according to the number of macro- and micro-metastases

| Type of metastases | Macrometastases | Micrometastases |
|---|---|---|
| Polymetastatic disease | ++ | ++/+ |
| Oligometastatic disease | + | +/− |
| Argometastatic disease<br>(True oligometastatic disease) | + | − |

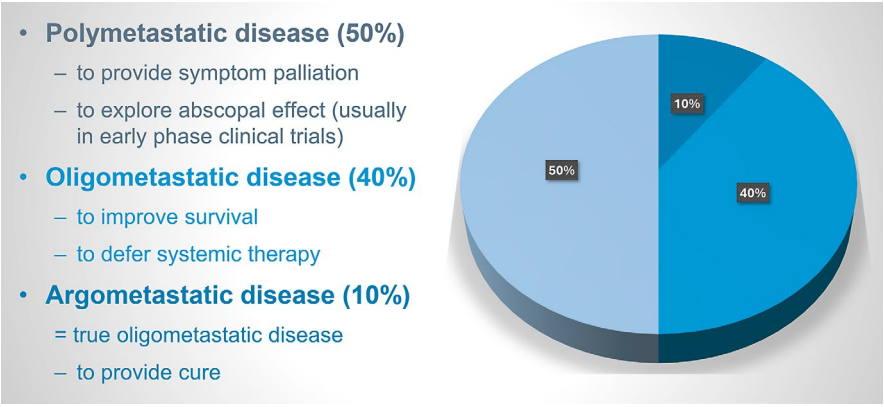


**Fig. 24.4** The utility of local therapy in the management of distant metastases in head and neck cancer according to the type of distant metastases. The percentages were calculated from datasets encompassing different tumor types

Of note, the concept of argometastases, albeit supported by a strong rational with both prospective and retrospective components, remains hypothetical and needs to be confirmed in dedicated clinical trials.

## Local therapy of the Primary Tumour in the Presence of Distant Metastases

Due to emerging new data, this intriguing topic was only recently discussed at the last THNO meeting and will therefore be presented in this chapter. Local tumour progression in the head and neck region can have devastating consequences, both in terms of survival and quality of life. Despite recent advances in systemic therapy, marked by the introduction of immune checkpoint inhibitors, outcomes remain poor, and the vast majority of unselected patients will eventually present with progressive disease. However, mounting evidence suggests that SCCHN patients with distant metastases have improved overall survival and quality of life when systemic palliative treatment is combined with oncologically sufficient local therapy, as if there was no metastatic dissemination. This approach can be indicated in patients with newly diagnosed SCCHN with synchronous distant metastases (Type 1 according to Fig. 24.1), in those with locoregionally uncontrolled SCCHN with synchronous distant metastases (Type 3A), and in those with a controlled but persisting primary tumour and metachronous distant metastases belonging to Type 2. Supporting data originate from different sources corresponding to different levels of evidence and are summarized below.

Starting at the preclinical stage, it was shown that cytoreduction diminishes the overall tumour burden and may thus improve the efficacy of systemic treatment by

eliminating pre-existing treatment-resistant clones [11]. In metastatic nasopharyngeal carcinoma, a disease closely related to SCCHN, a randomized phase III trial demonstrated that adding intensity-modulated radiotherapy (IMRT) at a dose of up to 70 Gy to a chemotherapy doublet with high-dose cisplatin and 5-fluorouracil leads to a significant improvement in 24-month overall survival (76.4% versus 54.5%; hazard ratio 0.42, $p = 0.004$) with comparable haematological and gastrointestinal toxicities but with an obvious difference in IMRT-related adverse events, which were observed only in the experimental arm [12]. In SCCHN, this concept has been supported by five large national registry analyses [13–17]. For example, Zumsteg and co-workers analysed the National Cancer Data Based cohort of 3269 patients with metastatic SCCHN followed for a median of 51.5 months. The 2-year overall survival rate was significantly improved when systemic palliative treatment was combined with local therapy of the primary tumour (34.2% versus 20.6%; $p < 0.001$). Importantly, this benefit could be attributed only to high-intensity local treatment, i.e., oncologic resection or radiotherapy of at least 60 Gy. Lower-intensity local treatment was equal to no local treatment at all in terms of efficacy [14]. In 2019, Rambeau and co-workers published the results of a retrospective multicentre study of 65 patients with upfront metastatic SCCHN treated with the EXTREME regimen. Forty-one patients received locoregional radiotherapy, yielding a significantly longer median overall survival (16.1 versus 7.5 months; $p < 0.01$), particularly if radiotherapy was delivered as consolidation in patients with disease control after three to six cycles of the EXTREME regimen, compared to those who received radiotherapy prior to chemotherapy [18]. Finally, a single-centre retrospective study assessed the outcomes in 40 patients with metastatic SCCHN treated with either surgery or chemoradiotherapy on top of palliative systemic therapy. The median progression-free and overall survival rates of 8.6 and 14.2 months, respectively, compared favourably to historical controls. In addition, those receiving a schedule with an immune checkpoint inhibitor had a median overall survival of unprecedented 41.7 months, with a 5-year overall survival of 39%. On the other hand, no immunotherapy meant a reduction in median overall survival to 12.1 months [19].

Despite an obvious heterogeneity in the analysed studies, the potential beneficial effect of local therapy for the primary tumour in the presence of metastatic dissemination is unquestionable. However, standardisation is essential, although outcomes will heavily depend on the quality of local treatment. Some of the questions that need to be addressed comprise the timing of systemic treatment, the drugs used, the dose of radiotherapy, and the extent of surgery.

## Conclusions

In oncology, a substantial portion of research dedicated to innovation is linked to drug development, which, notwithstanding its undeniable benefits, requires high resource investments. We have shown that repurposing traditional anticancer modalities by changing their target indications brings substantial improvements. These

improvements are measurable using both standard efficacy endpoints and quality of life outcomes. Moreover, the described therapeutic approaches can be, to a certain extent, immediately applied in clinical practice, providing selected patients with the best care according to the most recent evidence.

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# Part IV
# Salivary Gland Cancer

# Chapter 25
# Molecular Characterization of Salivary Gland Cancer and Treatment Implications

**Alice Rossi and Alberto Hernando-Calvo**

## Introduction

Salivary gland cancers (SGC) are rare and heterogeneous entities encompassing more than 20 different histologic subtypes [1]. To date, there are no preferred standard-of-care regimens for recurrent or metastatic disease and systemic treatment may be individualized based on tumor histologic subtype, patient characteristics and treatment availability [2]. Chemotherapies and non-matched targeted agent options have demonstrated limited efficacy in the treatment of SGC. Platinum-based chemotherapy regimens (e.g. cisplatin/cyclophosphamide/doxorubicin, cisplatin/vinorelbine, carboplatin/paclitaxel, carboplatin/gemcitabine) have shown to be active only for a subset of SGC. Phase 2 trials showed an objective response rate (ORR) of 27% with the CAP regimen (cisplatin, doxorubicin and cyclophosphamide) [3] and an ORR of 24% with the carboplatin plus gemcitabine doublet in SGC [4]. Moreover, a phase 2 trial testing cisplatin plus vinorelbine reached an ORR of 44% in patients with advanced SGC [5], while a retrospective study described an ORR of 39% with the use of carboplatin plus paclitaxel [6]. Only modest activity has been demonstrated with tyrosine kinase inhibitors, such as lenvatinib, which was evaluated in two phase 2 trials revealing an ORR ranging from 11.5% to 15.6% in recurrent or metastatic adenoid cystic carcinoma (ACC) [7, 8]. The limited efficacy of these therapeutic approaches underscores the need for innovative strategies.

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Given the limited long-term benefit of chemotherapy or unmatched targeted therapies and the need for novel therapeutic options in SGC, comprehensive molecular profiling could help to unveil the presence of actionable targets for systemic therapies [9]. Next-generation sequencing (NGS) and immunohistochemistry (IHC) assays have enabled the discovery of novel targetable genomic alterations present across SGC subtypes [10]. Although the majority of SGC are typically defined according to histology and immunohistochemistry (IHC) findings, the addition of NGS represents a valuable tool to improve diagnostic work-up between morphologically similar entities [11]. Given the relatively large prevalence of actionable alterations in SGC and the increasing role of targeted therapies in the treatment of recurrent or metastatic SGC [12] the National Comprehensive Cancer Network (NCCN) guidelines now recommend the evaluation of human epidermal growth factor receptor 2 (HER2) and androgen receptor (AR) status by IHC, as well as the use of genomic profiling to detect alterations in neurotrophic tropomyosin receptor kinase (*NTRK*), Harvey rat sarcoma virus (*HRAS)* and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA)* and to determine tumor mutational burden (TMB) [2]. The ESMO-European Reference Network on Rare Adult Solid Cancers (EURACAN) guidelines suggest the use of NGS or whole genome sequencing (WGS) for all SGC subtypes, justified by the fact that actionable mutations can be identified in 40–50% of the cases [1]. Patients diagnosed with rare cancers, such as SGC, who get access to profiling technologies may have the possibility to receive effective matched therapies, in a field where no standard therapies have been recognized [13]. However, in clinical practice patient's access to molecularly-guided therapies is typically constrained and differences in access to biomarker testing and reimbursement strategies for targeted therapies have been reported across countries [14]. In the rapidly evolving field of precision oncology, tracking evidence from real-world settings and molecular tumor boards will be key to implement personalized treatment approaches in SGC [15]. In this review, we provide an overview of current precision oncology approaches for SGC, highlighting promising targets for molecularly-guided therapies, challenges to their implementation, and potential future directions.

## HER2

HER2 protein is encoded by the *ERBB2* (erb-2 receptor tyrosine kinase 2) gene. HER2 overexpression, gene amplification and pathogenic mutations have been identified across tumor types as potential actionable alterations [16]. Although specific SGC scoring systems have been proposed, HER2 positivity criteria according to breast cancer guidelines are the most commonly used to assess HER2 overexpression in SGC [2, 17]. According to a recent meta-analysis by Egebjerg et al., the prevalence of HER2 positivity evaluated by either IHC (reporting semiquantitative scores) or by ISH (quantitative ratios) is very heterogeneous across SGC subtypes, ranging from 0% to 43% of the cases [18]. The estimated prevalence is higher in

salivary duct carcinoma (SDC) (43%, 95%CI 36–51%), followed by carcinoma ex pleomorphic adenoma (39%, 95%CI 32–45), squamous cell carcinoma (17%, 95%CI 7.5–33), adenocarcinoma not otherwise specified (NOS) (13%, 95%CI 7.6–21), poorly differentiated carcinoma (6.7%, 95%CI 0.17–32), mucoepidermoid carcinoma (5.5%, 95%CI 2.9–9.6), and with lower frequency in myoepithelial carcinoma, epithelial-myoepithelial carcinoma, acinic cell carcinoma and ACC [18]. According to the NCCN and ESMO-EURACAN guidelines, it is recommended that patients with HER2 positive SGC may receive a HER2-targeted treatment option with treatment choice individualized based on patient characteristics [1, 2].

Different prospective clinical trials have evaluated the role of anti-HER2 therapies in SGC (Table 25.1). A phase 2 Japanese study investigating trastuzumab plus docetaxel in 57 patients with advanced HER2-positive SGC showed an ORR of 70.2% (95%CI, 56.6–81.6). At the data cut-off with a median follow-up of 28 months, the median progression-free survival (PFS) was 8.9 months, and the median overall survival (OS) was 39.7 months [19]. In the phase 2 basket trial "MyPathway", the combination of trastuzumab plus pertuzumab was tested in patients with tumors with HER2 amplification and/or overexpression and/or pathogenic mutations across different advanced tumor types. Among 15 patients with SDC with HER2 amplification/overexpression, 9 had responses with an ORR=60% and a median duration of response (DOR) of 9.2 months. Notably, disease stabilization was observed in a patient with *HER2* mutant SDC treated with pertuzumab and trastuzumab achieving a PFS of 11.0 months suggesting that not only HER2 amplification and/or overexpression may sensitize SGC to anti-HER2 therapies [12]. A phase 2 basket trial evaluated trastuzumab-emtansine (T-DM1) in solid tumors harboring HER2 amplification (defined as copy number higher than 7 based on Oncomine AmpliSeqTM panel NGS), an ORR of 90% (9/10; 95%CI 56–100) was reported, including five complete responses after prior trastuzumab, pertuzumab and anti-androgen therapy [20]. The NCI-MATCH trial (EAY131) subprotocol Q, testing T-DM1 in patients with HER2-amplified tumors (excluding breast and gastric cancer) did not meet the primary end point for ORR in the overall population, but clinically meaningful activity was observed in SGC with durable responses in two out of the three patients treated [21]. Encouraging results for trastuzumab deruxtecan (T-DXd) have also been described in SGC. Seventeen patients with HER2-positive SDC were pooled in the combined subgroup analysis of two phase 1 studies testing T-DXd, the first-in-human NCT02564900 (eight patients) and the drug-drug interaction NCT03383692 trials (nine patients). Interestingly, among 14 SGC patients treated with T-Dxd who received other HER2 targeted agents as a prior cancer therapy, there were 8 partial responses with a confirmed ORR of 47%; median DOR was 12.9 months and median PFS was 14.1 months [22]. The DESTINY-PanTumor02 phase 2 trial tested T-DXd in different cohorts of tumors (excluding breast, colorectal, gastric or lung tumors) with HER2 2+ or 3+ score by immunohistochemistry; an ORR of 37.1% was observed across tumor cohorts with responses observed in SGC patients. Responses were observed irrespective of prior HER2 therapy [23]. Based on the promising results of DESTINY-PanTumor02 (NCT04482309), DESTINY-Lung01 (NCT03505710), and DESTINY-CRC02

**Table 25.1** Summary of selected clinical trials testing HER2-guided therapies both in SGC specific protocols and all-solid malignancies

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Trastuzumab + Pertuzumab | NCT02091141 | My Pathway: A Study Evaluating Herceptin/ Perjeta, Tarceva, Zelboraf/Cotellic, Erivedge, Alecensa, and Tecentriq Treatment Targeted Against Certain Molecular Alterations in Participants With Advanced Solid Tumors | 2 | ORR | ORR = 60% (n = 9/15), data cutoff 15 January 2018 |
| Trastuzumab and Docetaxel | UMIN000009437 | Docetaxel and Trastuzumab therapy or maximal androgen blockade for patients with recurrent and/or metastatic salivary grand carcinoma | 2 | ORR | ORR = 70.2% |
| Trastuzumab deruxtecan (T-DXd) | NCT02564900 | Phase 1, Two-Part, Multicenter, Non-randomized, Open-label, Multiple Dose First-In-Human Study of DS-8201A, in Subjects With Advanced Solid Malignant Tumors | 1 | Safety and ORR | ORR = 47% (8/17) |
| | NCT03383692 | A Phase 1, Multicenter, Open-label, Single Sequence Crossover Study to Evaluate Drug-drug Interaction Potential of OATP1B/ CYP3A Inhibitor on the Pharmacokinetics of DS-8201a in Subjects With HER2-expressing Advanced Solid Malignant Tumors | 1 | | |

(continued)

**Table 25.1** (continued)

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Trastuzumab deruxtecan (T-DXd) | NCT04482309 | A Phase 2, Multicenter, Open-label Study to Evaluate the Efficacy and Safety of Trastuzumab Deruxtecan (T-DXd, DS-8201a) for the Treatment of Selected HER2 Expressing Tumors (DESTINY-PanTumor02) | 2 | ORR | n/a (response(s) reported also in salivary gland cancer) |
| Ado-Trastuzumab Emtansine | NCT02675829 | A Phase 2 Trial of Ado-Trastuzumab Emtansine for Patients With HER2 Amplified or Mutant Cancers | 2 | ORR | ORR = 90% (9/10) |
| Ado-Trastuzumab Emtansine | NCT02465060 | Ado-trastuzumab emtansine (T-DM1) in patients with HER2-amplified tumors excluding breast and gastric/gastroesophageal junction (GEJ) adenocarcinomas: results from the NCI-MATCH trial (EAY131) subprotocol Q | 2 | ORR | ORR = 66.7% (2/3) |
| Trastuzumab deruxtecan (T-DXd) | NCT04639219 | A study of T-DXd for the treatment of solid tumors harboring HER2- activating mutations (DPT01) | 2 | ORR | ORR = 66.7% (4/6) in the subgroup of salivary gland/head and neck adenocarcinoma |
| GQ1001 | NCT04450732 | Safety of GQ1001 in adult patients with HER2-positive advanced solid tumors | 1 | Safety | n/a |

*ORR* objective response rate, *n/a* not available

(NCT04744831) trials, T-DXd was granted accelerated approval by the U.S Food and Drug Administration (FDA) in April 2024 for patients with unresectable or metastatic HER2-positive (IHC3+) solid tumors who have received prior systemic treatment and have no satisfactory alternative treatment options [24]. Efficacy and safety of T-DXd in patients with solid tumors harboring specific HER2-activating mutations was also demonstrated by DESTINY-PanTumor01 study with an ORR of 29.4% in the general population; in the subgroup of SGC the ORR was 66.7% (n = 4/6 patients) [25].

Possible mechanisms of resistance to HER2 targeted therapies may include incomplete receptor inhibition, inefficient internalization, development of multi-drug resistance proteins that pump drugs out of the tumor cell or activation of bypass pathways [26]. *PIK3CA* mutations and tumor suppressor phosphatase and tensin homolog (*PTEN)* genomic loss can play a role in the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway activation and anti-HER2 resistance. Additional downstream pathways that may confer resistance to anti-HER2 therapies include other receptors or cellular tyrosine kinases, such as mesenchymal epithelial transition (MET) or insulin growth factor-1 (IGFR-1) [27]. Notably, Kirsten rat sarcoma (*KRAS)* co-mutations may contribute to resistance to anti-HER2 therapies. As reported in the "MyPathway" phase 2 trial, a numerically higher response rate was observed for patients with *KRAS* wild type as compared to those with *KRAS*-mutated cancers (ORR = 28.1% vs 7.1% respectively) [28]. Ongoing clinical trials are investigating new compounds for HER2-positive tumors specifically in the field of SGC: a phase 1 trial is testing the HER2-targeting antibody drug conjugate (ADC), GQ1001 (NCT04450732), while a trial testing a binary oncolytic adenovirus in combination with HER2-specific autologous chimeric antigen receptor (CAR) T cells is currently recruiting (NCT03740256). Interestingly, considering the efficacy observed for anti-HER2 therapies in the metastatic setting, a phase 2 study is also exploring the efficacy of T-DM1 in the adjuvant setting for HER2-positive SGC (NCT04620187).

## Androgen Receptor

AR is a nuclear steroid hormone receptor that binds to testosterone or 5α-dihydroxytestosterone. A significant number of advanced SGC are AR-positive. Indeed, AR positivity is reported in 78–96% of patients with SDC [9] and in 20–30% of adenocarcinoma NOS [29]. AR overexpression has also been reported in other histologic SGC subtypes, such as carcinoma ex pleomorphic adenoma, mucoepidermoid carcinoma and basal cell adenocarcinoma [30, 31]. In 2018, a Japanese phase 2 clinical trial evaluated the efficacy and safety of combined androgen blockade (CAB) with leuprorelin acetate and bicalutamide (either as first- or further lines

of treatment) in 36 AR-positive SGCs: 34 SDCs (94%) and 2 adenocarcinomas NOS (6%). The ORR was 41.7% (15/36), with a median PFS and OS of 8.8 and 30.5 months respectively and a tolerable safety profile [31]. In 2021, a phase 2 study investigated the efficacy of the combination of abiraterone plus a luteinizing hormone-releasing hormone (LHRH) agonist as a second line hormone-treatment after failure of androgen deprivation therapy (ADT) in 24 patients with castration-resistant, AR-positive SGC (19 with SDC and 5 with adenocarcinoma NOS). The ORR was 21% (5/24). The median PFS was 3.7 months and median OS 22.5 months [32]. The randomized, phase II EORTC HNCG/UKCRN trial is ongoing to evaluate the efficacy of ADT compared to chemotherapy as first line treatment in patients with advanced AR-positive SGC (NCT01969578).

Despite these promising trends, a phase 2 trial investigating enzalutamide as monotherapy in AR-positive locally advanced/unresectable or metastatic SGC (NCT02749903) showed a confirmed ORR of 4.3% (2/46), failing to meet the primary endpoint of ORR. The trial enrolled 46 patients, of which 30 (65.2%) had received prior systemic therapy, including 13 treated with AR-targeted drugs. A median PFS of 5.6 months was observed and 39.1% of patients (18/46) were progression-free for at least 6 months, suggesting the potential clinical benefit of enzalutamide in terms of disease control [30]. Indeed, an unplanned analysis showed that the 6-month clinical benefit rate (defined as complete response + partial response + stable disease) was higher among patients with more than 70% AR-positive tumor cells (52.0% vs 20.0%; $p = 0.0045$) and lacking HER2 overexpression/amplification (45.8% vs 22.2%; $p = 0.0126$) [30]. Currently, ongoing clinical protocols encompass trials testing combinations of treatments, in order to overcome resistance mechanisms and enhance efficacy. A phase 2 clinical trial is investigating the addition of dutasteride to CAB therapy in advanced SDC (NCT05513365). A phase 2 study of darolutamide plus leuprolide acetate in hormone therapy naive AR-positive SGC is ongoing (NCT05669664) (Table 25.2). The co-expression of HER2 and AR has been observed in SGC, specially in 35–58% of SDC patients. Currently, in case of positivity for both markers there is no sufficient evidence to support target prioritization between HER2 or a AR-targeted therapy [32]. In these cases, treatment selection may rely in treatment availability, toxicity profile or patients preferences.

Different mechanisms of resistance have been explored in AR-positive SGC exposed to CAB. The expression of the splice variants, for example ARv7, known to be implicated in resistance to ADT in prostate cancer, could be exploited to predict the onset of resistance in SDC. Liquid biopsy may serve as a functional tool to investigate resistance mechanisms [33]. In a cohort of 89 patients with SDC treated with AR targeted therapy, higher expression of enhancer of zeste homolog 2 (EZH2) correlated with a lower ORR and a shorter OS, suggesting that a combination treatment targeting EZH2 and AR might overcome resistance of AR-targeted therapies in SDC patients [34].

**Table 25.2** Summary of selected clinical trials testing AR-guided therapies in SGC

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Leuprorelin acetate + bicalutamide | UMIN000009437 | Docetaxel and Trastuzumab therapy or maximal androgen blockade for patients with recurrent and/or metastatic salivary grand carcinoma | 2 | ORR | ORR = 41.7% (15/36) |
| Abiraterone + LHRH agonist | NCT02867852 | A Randomized Phase II Study to Evaluate the Efficacy and Safety of Chemotherapy (CT) vs Androgen Deprivation Therapy (ADT) in Patients With Recurrent and/or Metastatic, Androgen Receptor (AR) Expressing, Salivary Gland Cancer (SGCs) | 2 | ORR | ORR = 21% (5/24) |
| ADT | NCT01969578 | Androgen deprivation therapy in advanced salivary gland cancer | 2 | PFS (cohort A), BOR (cohort B) | n/a |
| Enzalutamide | NCT02749903 | Enzalutamide for patients with androgen receptor positive salivary cancers | 2 | BOR | ORR = 4.3% (2/46) in AR-positive SGC |
| Dutasteride + CAB therapy | NCT05513365 | Dutasteride in combination with CAB vs CAB in SDC | 2 | ORR, DOR | n/a |
| Darolutamide + leuprolide acetate | NCT05669664 | A Phase 2 study of darolutamide in people with testosterone-driven salivary gland cancer | 2 | BOR | n/a |

*ORR* objective response rate, *BOR* best overall response, *DOR* duration f response, *PFS* progression free survival, *n/a* not available

## *NTRK* Fusions

*NTRK* genes encode a family of receptor tyrosine kinases involved in neuronal development [35]. The phosphorylation of the intracellular domain of tropomyosin receptor kinase (TRK) receptors activates multiple cellular signaling pathways [36]. Chromosomal rearrangements involving the *NTRK1, NTRK2* and *NTRK3* genes have been identified across solid tumors. *NTRK* gene fusions are found in approximately 1% of all solid tumors, with a higher frequency in SGC [37]. Indeed, the

gene fusion *ETV6-NTRK3* can be considered almost pathognomonic in mammary analogue secretory carcinoma (MASC) with 95–98% of the cases harboring the recurrent balanced chromosomal translocation t(12;15)(p13;q25); the remaining 2–5% of the cases of rearrangements may involve *ETV6* and a non-*NTRK3* gene [11]. So far, the FDA has approved two TRK inhibitors, larotrectinib and entrectinib, for the treatment of *NTRK* fusion-positive tumors with a tumor-agnostic indication [38, 39]. Larotrectinib received tumor-agnostic FDA approval in 2018, based on data collected from 3 clinical trials: a phase 1 study involving adults (NCT02122913), a phase 1/2 study involving children (NCT02637687) and a phase 2 study involving adolescents and adults (NCT02122913). In 55 patients with *NTRK* fusion-positive solid tumors, an ORR of 75% was reached [40]. Specifically in SGC, at the data cut-off of 20 July 2020, 24 patients with TRK fusion-positive SGC treated with larotrectinib were identified from 2 clinical trials, NCT02122913 and NCT02576431; SGC histologies were mainly represented by secretory carcinoma (54%, 13/24), followed by adenocarcinoma NOS (25%, 6/24), mucoepidermoid carcinoma (13%, 3/24), ACC (4%, 1/24) and glandular sarcomatoid carcinoma (4%, 1/24). An ORR of 92% was observed, both in MASC (ORR = 85%) and in other SGC (ORR = 100%) [41]. Entrectinib, a pan-TRK inhibitor with additional activity against *ROS1* and *ALK* fusions, was approved by the FDA with a tissue-agnostic indication in 2019 for patients older than 12 years old with *NTRK* fusion-positive advanced solid tumors [39]. A pooled analysis from a phase 2 trial (STARTRK-2) and two phase 1 trials (STARTRK-1 and ALKA-372–001) analyzed the ORR of entrectinib in 54 patients treated with advanced or metastatic NTRK fusion-positive solid tumours, of which 13% (7/54) were MASC. The pooled analysis showed an ORR of 57% [42]. To date, no prospective head to head comparisons have been performed between larotrectinib and entrectinib.

Next-generation TRK inhibitors have been developed to overcome resistance to the "on-target" mutations which represent the most frequent resistance mechanisms to the first generation of TRK inhibitors in solid tumors, including substitutions in the solvent-front position (*NTRK1* G595R or *NTRK3* G623R), in the gatekeeper position (*NTRK1* F589L) and in the xDFG position (*NTRK1* G667S or *NTRK3* G696A) [40]. As an example of "on-target" resistance in a patient diagnosed with MASC who progressed to entrectinib after nearly 2 years of treatment, an *NTRK3* G623R mutation was identified experiencing an 8-month-long response after exposure to the second-generation TRK inhibitor selitrectinib [43]. Although *NTRK* fusions do not typically co-occur with other actionable genomic alterations [44], "off-target" mechanisms of acquired resistance due to bypass signaling pathway activation may be identified at the level of RAF-MEK-ERK signaling, such as *BRAF* V600E, *KRAS* G12A and *MET* amplifications suggesting a role for longitudinal molecular testing and possibly for novel combination treatment strategies [41, 45]. Due to these relevant data and to the histology-agnostic approvals of larotrectinib and entrectinib, nowadays the analysis of *NTRK* fusions is recommended for all SGC subtypes given the potential clinical impact of this intervention [1] (Table 25.3).

**Table 25.3** Summary of selected clinical trials testing NTRK inhibitors both in SGC specific protocols and all-solid malignancies

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Larotrectinib | NCT02122913 | A Study to Test the Safety of the Investigational Drug Larotrectinib in Adults That May Treat Cancer | 1 | Safety and ORR | n/a |
| | NCT02637687 | SCOUT: A Study to Test the Safety and Efficacy of the Drug Larotrectinib for the Treatment of Tumors With *NTRK*-fusion in Children | 1, 2 | Safety and ORR | |
| | NCT02122913 | NAVIGATE: A Study to Test the Effect of the Drug Larotrectinib in Adults and Children With *NTRK*-fusion Positive Solid Tumors | 2 | ORR | |
| Larotrectinib | NCT02122913 | A Study to Test the Safety of the Investigational Drug Larotrectinib in Adults That May Treat Cancer | 1 | Safety and ORR | Patients: 24 patients with *TRK* fusion-positive salivary gland cancer (cut-off July 20, 2020) ORR = 92% (22/24): CR = 13% (3/24), PR 79% (19/24), PD 8% (2/24) |
| | NCT02576431 | NAVIGATE: A Study to Test the Effect of the Drug Larotrectinib in Adults and Children With *NTRK*-fusion Positive Solid Tumors | 2 | ORR | |

(continued)

**Table 25.3** (continued)

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Entrectinib | 2012–000148–88 | ALKA-372-001: First-in-human, phase I dose-escalation study of entrectinib (RDXD-101)—an oral pan-trk, ROS1, and ALK inhibitor—in patients with advanced solid tumors with relevant molecular alterations | 1 | Safety | n/a |
| | NCT02097810 | STARTRK-1: Study of Oral RXDX-101 in Adult Patients With Locally Advanced or Metastatic Cancer Targeting NTRK1, NTRK2, NTRK3, ROS1, or ALK Molecular Alterations | 1 | Safety | |
| | NCT02568267 | STARTRK-2: Basket Study of Entrectinib (RXDX-101) for the Treatment of Patients With Solid Tumors Harboring *NTRK* 1/2/3 (Trk A/B/C), *ROS1*, or *ALK* Gene Rearrangements (Fusions) | 2 | ORR and DOR | |
| Selitrectinib | NCT03215511 | A Phase 1 Study of the TRK Inhibitor Selitrectinib (BAY 2731954) in Adult and Pediatric Subjects With Previously Treated *NTRK* Fusion Cancers | 1 | Safety | n/a |
| Repotrectinib | NCT03093116 | TRIDENT-1: A Study of Repotrectinib (TPX-0005) in Patients With Advanced Solid Tumors Harboring *ALK*, *ROS1*, or *NTRK* 1–3 Rearrangements | 1, 2 | Safety and ORR | n/a |
| Repotrectinib | NCT04094610 | A Study of Repotrectinib in Pediatric and Young Adult Subjects Harboring *ALK*, *ROS1*, OR *NTRK* 1–3 Alterations | 1, 2 | Safety and ORR | n/a |

(continued)

**Table 25.3** (continued)

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Taletrectinib | NCT04617054 | A Phase II, Multicenter, Open, Basket Study of AB-106 to Treat the Subjects With Local Progression or Systemic Metastasis Solid Tumors With *NTRK* Gene Fusion | 2 | ORR | n/a |

*ORR* objective response rate, *DOR* duration of response, *n/a* not available, *CR* complete response, *PR* partial response, *PD* progressive disease

## *RET* Fusions

Gain-of-function chromosomal rearrangements or mutations in the protooncogene *RET* (Rearranged During Transfection) are implicated in a broad spectrum of cancers through activation of several cellular pathways (e.g. RAS-MAPK, PI3K-AKT, JAK-STAT, PKC) [46, 47]. *RET* fusions are observed in less than 1% of SGC [48]. Case reports have described RET fusions in MASC, including ETV6-RET or VIM-RET fusions [49]. RET rearrangements have also been documented in intraductal carcinoma of the salivary glands (previously called low-grade cribriform cystadenocarcinoma) [50]. In September 2022, selpercatinib, a small molecule inhibitor of the RET receptor tyrosine kinase, received FDA tumor-agnostic approval for adult patients with advanced or metastatic *RET* fusion-positive solid tumors, based on data from the phase 1/2 LIBRETTO-001 clinical trial, in which an ORR = 43.9% was observed in 45 patients with *RET* fusion-positive solid tumors. The LIBRETTO-001 included four patients with SGC showing an ORR of 50% by independent review committee assessment [51]. The RET inhibitor pralsetinib, despite not being currently approved as a tumor-agnostic treatment, has demonstrated durable responses in the ARROW phase 1/2 trial for *RET*-mutated solid tumors with an ORR of 57%. Only one patient with SGC SDC was included in the study [48] (Table 25.4).

On the one hand, mechanisms of on-target resistance have been identified, including acquired secondary *RET* solvent front mutations. These mutations can directly interfere with the ability of RET inhibitors to bind to the RET protein effectively, reducing their inhibitory activity [52]. On the other hand, MAPK pathway activation secondary to *KRAS*, *NRAS*, *BRAF* mutations, *MET* mutations or *FGFR1* (*fibroblast growth factor receptor 1*) amplification may lead to bypass signaling resistance [53]. Current efforts are directed towards the development of the next-generation RET inhibitors [52], for example the next-generation RET inhibitor LOXO-260 in the ongoing phase 1 trial NCT05241834 has been designed to have activity against solvent front and gatekeeper mutations in *RET* fusion-positive advanced solid tumors [54].

**Table 25.4** Summary of selected clinical trials testing RET inhibitors both in SGC specific protocols and all-solid malignancies

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Selpercatinib | NCT03157128 | LIBRETTO-001: A Study of Selpercatinib (LOXO-292) in Participants With Advanced Solid Tumors, RET Fusion-Positive Solid Tumors, and Medullary Thyroid Cancer | 1, 2 | Safety and ORR | ORR = 50.0% in *RET* fusion-positive salivary gland tumors |
| Pralsetinib | NCT03037385 | ARROW: A Phase 1/2 Study of the Highly-selective RET Inhibitor, BLU-667, in Patients With Thyroid Cancer, Non-Small Cell Lung Cancer (NSCLC) and Other Advanced Solid Tumors | 1, 2 | Safety and ORR | n/a |
| LOXO-26 | NCT05241834 | A Phase 1 Study of Oral LOXO-260 in Patients With RET Fusion-Positive Solid Tumors, Medullary Thyroid Cancer, and Other Tumors With RET Activation Refractory to Selective RET Inhibitors | 1 | Safety and ORR | n/a |

*ORR* objective response rate, *n/a* not available

## *BRAF* Mutations

Potential targetable alterations in SGC include genomic alterations in *BRAF* (B-Raf proto-oncogene, serine/threonine kinase), which, although infrequent, have been reported in 0–5% of cases per histologic subtype (overall 2.7%) [55]. *BRAF* is a key regulator of the RAS/RAF/MEK/ERK signaling pathway for tumorigenesis, and the *BRAF* V600E missense mutation results in constitutive kinase activation, leading to downstream cancer cell proliferation [56]. On June 2022, the combination of dabrafenib and trametinib was FDA-approved with a tumor-agnostic indication for patients with unresectable or metastatic solid tumors harboring *BRAF* V600E mutations (except for colorectal cancer) [57]. The approval of dabrafenib and trametinib was based on a pooled analysis of 167 patients with *BRAF* V600 mutations enrolled across three multi-cohort trials: 131 adults from the BRF117019 (NCT02034110) and NCI-MATCH (NCT02465060) plus 36 pediatric patients enrolled in the CTMT212X2101 study (NCT02124772); these studies enrolled patients encompassing 24 different tumor types, with a global ORR of 54% [57]. Specifically in SGC, a case report supported the use of combined BRAF and MEK inhibition with dabrafenib and trametinib for a *BRAF* mutated metastatic SDC achieving a durable response [58]. Moreover, in the phase 2 basket trial "MyPathway", one patient with

mucoepidermoid carcinoma harboring a *BRAF* V600E mutation was treated with vemurafenib, a BRAF inhibitor, obtaining a partial response and a duration of response of 15.1 months and a PFS of 18.5 months [12]. (Table 25.5) Different

**Table 25.5** Summary of selected clinical trials testing BRAF inhibitors both in SGC specific protocols and all-solid malignancies

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Dabrafenib + Trametinib | NCT02034110 | Efficacy and Safety of the Combination Therapy of Dabrafenib and Trametinib in Subjects With BRAF V600E- Mutated Rare Cancers | 2 | ORR | n/a |
| Dabrafenib + Trametinib | NCT02034110 | Efficacy and Safety of the Combination Therapy of Dabrafenib and Trametinib in Subjects With BRAF V600E- Mutated Rare Cancers | 2 | ORR | n/a |
| | NCT02465060 | NCI-MATCH trial subprotocol H included 35 patients with BRAF V600 mutated different tumor types (except for melanoma, CRC, and thyroid cancers) | 2 | ORR | |
| | NCT02124772 | MEK Inhibitor Trametinib in Children and Adolescents Subjects With Cancer or Plexiform Neurofibromas and Trametinib in Combination With Dabrafenib in Children and Adolescents With Cancers Harboring V600 Mutations | 1, 2 | Safety and ORR | |
| MEK and panRAF inhibitor | NCT05564377 | Subprotocols (EAY191-C1), a combination of a MEK inhibitor and a panRAF inhibitor in Molecular Analysis for Combination Therapy Choice (ComboMATCH) trial | 2 | ORR | n/a |
| Vemurafenib | NCT02091141 | My Pathway: A Study Evaluating Herceptin/ Perjeta, Tarceva, Zelboraf/ Cotellic, Erivedge, Alecensa, and Tecentriq Treatment Targeted Against Certain Molecular Alterations in Participants With Advanced Solid Tumors | 2 | ORR | ORR = 100% (n = 1/1), data cutoff 15 January 2018 |

*ORR* objective response rate, *n/a* not available

mechanisms of resistance have been reported for BRAF V600 inhibitors which include both acquired secondary resistance mutations and paradoxical hyperactivation of the MAPK cascade [59]. By targeting the dimer interface of the BRAF kinase domain, allosteric BRAF inhibitors may overcome resistance acting as "paradox breakers" [60].

## Immune Checkpoint Inhibitors

Immunotherapy harnesses the host immune system to identify and eradicate cancer cells [61]. The disruption in the clinic of checkpoint inhibitors targeting the PD-1 (programmed death-1)/PD-L1 (programmed death-ligand 1) axis has dramatically changed the treatment landscape across tumor types [62, 63]. In April 2020, based on the results from the KEYNOTE-158 clinical, the FDA granted priority review for the anti-PD-1 monoclonal antibody pembrolizumab for patients with advanced solid tumors with high TMB, defined as ≥10 mutations per megabase as assessed by an FDA-approved test [64, 65]. Pembrolizumab was the first drug to be approved with a tumor-agnostic indication in May 2017 for the treatment of microsatellite instability-high solid tumors, based on pooled efficacy data from 149 patients enrolled across five multi-cohort, non-randomized clinical trials: KEYNOTE-016, KEYNOTE-164, KEYNOTE-158, KEYNOTE-012 and KEYNOTE-028; the integrated ORR of pembrolizumab for solid tumors was 39.6% [66]. In the KEYNOTE-158 trial, 109 patients with advanced SGC were treated with pembrolizumab, with an ORR of 4.6% (5/109) in the overall population irrespective of PD-L1 expression. In this study, PD-L1 positivity was assessed using the combined positive score (CPS) ≥1, evaluated with PD-L1 IHC 22C3 pharmD. When stratifying by the CPS score, ORR was 10.7% for PD-L1–positive patients and 2.6% for PD-L1 negative. Across 3 patients with TMB-High SGC, one patient with both Microsatellite Instability/Mismatch Repair–Deficient (MSI/dMMR) and TMB-high disease experienced a response, demonstrating the limited but documented efficacy of pembrolizumab in SGC [67]. In this regard, the immune checkpoint inhibitor pembrolizumab could be considered as a therapeutic option for patients with TMB-high or microsatellite instability (MSI)/deficient mismatch repair (dMMR) advanced/metastatic SGC [64]. Efficacy of the anti-PD-1 drug nivolumab was evaluated in the "NISCAHN" phase 2 trial, where the primary endpoint of nonprogression rate at 6 months was met in the ACC cohort (33% nonprogressive patients at 6 months), while efficacy in the non-ACC cohort failed to be demonstrated [68]. Although novel combination regimens with immune checkpoint inhibitors have been attempted in SGC to improve their therapeutic effects, limited efficacy has been observed so far. A phase II trial evaluated the anti-PD-1 nivolumab and the anti-CTLA4 (cytotoxic T-lymphocyte antigen 4) ipilimumab in 64 patients with advanced SGC (32 ACCs in cohort 1 and 32 with other SGC subtypes in cohort 2). This study showed limited efficacy in ACC patients with an ORR = 6% in cohort 1,

while a relatively higher ORR of 16% was identified in the non-ACC cohort 2, worth for further investigation [69].

Given the low prevalence of SGC, interpretation of the current results is limited by single-arm study designs and the lack of appropriate comparator arms [67]. As only few patients with SGC demonstrate benefit to immunotherapy, current research focuses on the understanding of sensitivity or resistance mechanisms and the development of novel combination strategies to improve their effectiveness [68]. Ongoing trials are evaluating whether combining pembrolizumab with other agents can improve outcomes among patients with SGC. Two clinical trials combining immunotherapy and cytotoxic chemotherapy are ongoing. A phase II clinical trial using the combination of pembrolizumab and docetaxel in salivary gland and thyroid cancers is ongoing (NCT03360890). An additional phase 2 clinical trial is investigating the combination of pembrolizumab and pemetrexed for SGC (NCT04895735). Lastly, a phase 2 trial is currently testing the combination of ADT and pembrolizumab for AR-positive SGC (NCT03942653) (Table 25.6).

**Table 25.6** Summary of selected clinical trials testing checkpoint inhibitors both in SGC specific protocols and all-solid malignancies

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Pembrolizumab | NCT02628067 | KEYNOTE-158: Study of Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors; Evaluation of pembrolizumab monotherapy in patients with previously treated advanced salivary gland carcinoma in the phase 2 KEYNOTE-158 study | 2 | ORR | ORR = 4.6% |
| Nivolumab | NCT03132038 | Nivolumab in recurrent or metastatic Salivary Gland Carcinoma of the Head and Neck (NISCAHN) | 2 | Non-progression rate at 6 months | Non-progression rate at 6 months = 15/45 (33.3%) for ACC; 7/50 (14.0%) for non-ACC |
| Nivolumab + Ipilimumab | NCT03172624 | Study of Nivolumab plus Ipilimumab in patients with Salivary Gland Cancer | 2 | ORR | ORR = 2/32 (6%) in ACC cohort, ORR = 16% (5/32) in non-ACC cohort |

(continued)

**Table 25.6** (continued)

| Molecular targets/Drugs | Trial ID | Description | Phase | Primary endpoint | Efficacy in SGC |
|---|---|---|---|---|---|
| Pembrolizumab + docetaxel | NCT03360890 | Pembrolizumab with chemotherapy for poorly chemo-responsive Thryroid and Salivary Gland Tumors (iPRIME) | 2 | ORR | n/a |
| Pembrolizumab + pemetrexed | NCT04895735 | Pemetrexed and Pembrolizumab for the treatment of recurrent and/or metastatic Salivary Gland Cancer | 2 | ORR | n/a |
| Pembolizumab + ADT | NCT03942653 | Androgen Deprivation Therapy (ADT) and pembrolizumab for advanced stage Androgen Receptor positive Salivary Gland Carcinoma | 2 | ORR | n/a |

*ORR* objective response rate, *n/a* not available

## Current Challenges and Future Directions

Chemotherapy and non-matched targeted agents have demonstrated limited efficacy in SGC [1, 2]. Currently, molecular profiling in advanced or metastatic SGC is recommended by international guidelines, since therapeutic implications may be beneficial for patients. In the near future, novel actionable targets will probably define even smaller biomarker-based populations across SGC subtypes. Moreover, molecular profiling may also help identify mechanisms of response or resistance to targeted therapies. In this regard, access to comprehensive genomic profiling may become key for the detection and improvement of diagnostic work up [33, 34]. In this review, we discussed molecularly-guided therapeutics already recommended by current SGC guidelines as well as novel promising targeted agent candidates, both with specific interest in SGC or as tumor-agnostic agents. To date, how to generate proof of activity for molecularly-guided therapies in rare cancers, including SGC, remains an open question. The analysis of data from trials enrolling patients with SGC is typically constrained by small and heterogeneous cohorts. The relatively low efficacy of existing therapies for advanced SGC may be overcome by deepening the molecular understanding of the tumor biology to identify the presence of co-drivers. Longitudinal evaluation of molecular profiles in SGC may became part of the standard management to clarify mechanisms of primary and secondary resistance to targeted agents [70].

To overcome the limitations associated with the low prevalence of SGC and its impact on clinical trial enrollment, novel clinical trial designs such as master protocols, categorized as basket, umbrella or platform trials, may represent a unique opportunity for individuals with SGC [70]. Besides the use of DNA-based NGS techniques, the integration of RNA-based technologies in clinical trials may provide a unique opportunity for novel target discoveries to improve patient selection [71]. International collaboration among academic institutions will be essential to collect data and develop the next generation of clinical trials that allow inclusion of SGC patients. The analysis of administrative data associated with diagnostic or therapeutic interventions may help us to elucidate cost-effectiveness for reimbursement strategies in public health systems to implement homogenous biomarker strategies across countries [72]. Considering the relatively high rate of actionable alterations observed across SGC subtypes, the downstream effects of NGS (e.g. impact on clinical outcomes and cost-effectiveness) may increase with the breadth of genomic alterations analyzed and targeted therapies availability. The next generation of clinical trials may include patient-centered approaches to evaluate customized treatment combinations tailored for each tumor molecular profiles [70, 73]. N-of-1 trials aim to match drugs to individual patients [74, 75] and may emerge as a potential flexible tool for drug development in ultra rare cancers such as specific SGC subtypes [76].

While chemotherapy and unmatched targeted therapies offer only limited long-term benefits, the use of molecular profiling in solid tumors and SGC reveals actionable alterations and prognostic insights. Matching patients' treatments with their molecular alterations may be associated with improved outcomes, but further efforts will be required to implement precision oncology approaches in the therapeutic management of SGT with the ultimate goal of improving patient outcomes and quality of life.

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# Chapter 26
# Salivary Duct Carcinoma: Strategies to Improve Outcome for Recurrent/ Metastatic Disease

**Stefano Cavalieri and Lisa Licitra**

## Background

Salivary Gland Cancer (SGC) are rare malignant tumors with a relevant cancer-specific mortality, with a 5-year relative survival of 63% [1]. SGCs encompass more than 20 pathologic entities with specific biological and clinical behaviors.

Targeted treatments have revolutionized the disease in some SGCs. Among actionable targets, the human epidermal growth factor receptor-2 (HER2) is a well-characterized proto-oncogene. HER2 overexpression and amplification play a crucial role in breast and gastric cancer. However, its role has only recently become evident in SGC.

HER2 aberrations may be found in several high-grade SGCs with variable rates according to the pathologic subtype. HER2 alterations are constantly absent in low-grade and indolent SGCs. High-grade tumors, especially salivary duct carcinoma (SDC) and adenocarcinoma not otherwise specified (NOS), are characterized by higher frequencies of HER-2 gene alterations (32% SDC, 15–17% adenocarcinoma NOS, carcinoma ex pleomorphic adenoma 32%) [2].

HER2-overexpressing and amplified SGCs should be regarded as different entities from HER2-negative ones. Mimicking the classification of breast cancers (hormone-positive, HER2-negative; hormone-positive, HER2-positive; hormone-negative, HER2-positive; triple-negative disease, meaning hormone-negative, HER2-negative), a similar grouping has been proposed for SDC. Five groups were proposed: apocrine A and B (androgen receptor [AR]-positive, HER2-negative, with low and high ki67, respectively), apocrine HER2 (AR-positive,

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HER2-positive), HER2-enriched (AR-negative, HER2-positive), double negative (AR-negative, HER2-negative) [3, 4].

Independent of the underlying biological mechanism leading to HER2 amplification, HER2-positive SGC patients have a worse prognosis than their HER2-negative counterparts [3, 4]. In retrospective studies, HER2-overexpression was unrelated to worse prognosis [5], including a large retrospective case series SDC and adenocarcinoma NOS patients treated in a tertiary cancer center in the USA [6]. On the other hand, other studies focusing on recurrent/metastatic (R/M) AR-positive SGC patients only showed the negative prognostic role of HER2 [7].

The former study included 200 patients (110 SDC, 90 adenocarcinoma NOS) accessing the MD Anderson Cancer Center (MDACC) in Houston, US, between 1990 and 2020. Main inclusion criteria were: having accessed the head and neck department at least twice; centralized pathology review by expert pathologist; availability of clinico-pathological characteristics, known treatments, and outcome data. The majority (61%) of the study patients had stage III-IVb disease (69.9% in the SDC cohort). The prevalence of subjects with upfront stage IVC disease were 13% and 9.1% in the overall case series and in the SDC subgroup, respectively. The median recurrence-free survival was 2.05 years in the overall study population, 1.61 years in the SDC cohort.

The Italian study included patients with R/M AR-positive SGC only accessing the Fondazione IRCCS Istituto Nazionale dei Tumori in Milan, Italy, between 2010 and 2021. Main inclusion criteria were: availability of HER2 status; centralized pathology review; availability of clinical, pathological and survival data. A total of 74 R/M AR-positive SGC patients were included, of whom 51 had SDC (82%). The disease-free interval was 16.3 months in the overall population, 12.1 months in the HER2-positive cohort, and 19.2 months in the HER2-negative one.

Although no direct comparisons can be made between retrospective case series published at different institutions, the American and European studies differed for several reasons. In the US study, only 77% of patients had AR-positive disease, and all disease stages were included. This means that patients with potentially curable disease at diagnosis or with metastatic disease upfront were also included. In the Italian case series, only patients with R/M disease were included. Approximately one third (13 cases) of them were diagnosed with a de novo metastatic disease and did not received loco-regional treatment upfront.

Only patients affected by R/M SDCs are usually treated with antiandrogen or anti-HER2 agents, except for patients receiving off-label adjuvant therapy after locoregional treatments.

Moreover, the American study classified tumors as HER-2 positive if they scored 2+ or 3+ by immunohistochemistry (IHC), regardless of in-situ hybridization (ISH) confirmation [6]. The Italian study included only patients with R/M AR-positive disease, and tumors were classified as HER2-positive only if the IHC score was 3+ or 2+ with gene amplification by ISH.

Even the threshold for AR positivity was different in the two case series. In the former, positivity was defined as IHC staining in $\geq$10% of tumor cells, in the latter as a combined expression score obtained by summing the scores of staining

intensity and extent. This difference implied a population with higher expression of AR and HER2 in the European study.

Finally, a lower prevalence of patients treated with anti-HER2 was observed in the Italian cohort (27%) compared to the American experience. In the US study, 53% of HER2+ SGC patients requiring a first-line systemic therapy were treated with at least one anti-HER2-based regimen (10 in the first line) [6]. Likely, the different regulatory and reimbursement agencies and laws between the US and European Countries might have impacted this different prevalence.

Given the differences between HER2-positive and negative SDCs, strategies to improve outcomes of R/M SDC patients should consider the following combinations of disease presentation based on AR and HER2 expression.

## *AR-Positive HER2-Negative SDC*

Androgen deprivation therapy (ADT) has been assessed in R/M AR-positive SGCs, with AR positivity defined by staining solid intensity (score 3/3) and staining extent of ≥70% immunopositive nuclei score [8].

In a retrospective case series published in 2016, 17 SGC patients (8 SDC, 9 NOS) were treated with combined ADT. The combined blockade with bicalutamide 50 mg once a day and triptorelin 3.75 mg once a month provided an objective response rate (ORR) of 65% (50% in the SDC subgroup) [8]. Out of the 3 patients achieving a complete response (CR), two had SDC. A wider cohort of patients (36 subjects, 34 with SDC) enrolled in a phase II study showed a lower ORR (42%), with 4 subjects obtaining a complete response [9]. In 2021, a phase II study enrolling 24 patients (19 SDC) demonstrated that abiraterone is a safe and active chemo-free therapeutic option for R/M AR-positive SGC patients after ADT failure, independently of previous chemotherapy (CT) [10].

The EORTC 1206 clinical study was a randomized phase II trial (Table 26.1) designed to assess the best first-line approach for AR-positive R/M SGC patients [11]. Patient recruitment has been concluded, but the final results are not available

**Table 26.1** Study design of the EORTC1206 clinical trial

| AR | HER2 | Treatment |
|---|---|---|
| Positive | Positive | ADT (e.g., monthly LHRH analog + daily bicalutamide) and/or CT (e.g., taxanes ± platinum salts) and/or anti-HER2 (e.g., trastuzumab) |
| Positive | Negative | ADT (e.g., monthly LHRH analog + daily bicalutamide) and/or CT (e.g., platinum salts + taxanes or anthracyclines) |
| Negative | Positive | Anti-HER2 +/− CT<br>(e.g, docetaxel + trastuzumab; TDM1; trastuzumab-deruxtecan) |
| Negative | Negative | CT (e.g., platinum salts + taxanes or anthracyclines); consider molecular characterization |

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yet. Until the publication of that study, information about the most effective first-line systemic option for R/M AR-positive SDC patients is missing.

Independent of this clinical study, for AR-positive HER2-negative SGC patients, there is still an urgent need to explore new-generation androgen blockade strategies, which may include combinations of hormone treatments and/or CT. Recent evidence on metastatic hormone-sensitive prostate cancer patients showed that adding the antiandrogen darolutamide to ADT and CT improves overall survival over ADT + CT [12]. Moreover, the association of taxane-based CT and ADT does not increase toxicities when compared to ADT alone in prostate cancer patients [13].

Thus, exploring similar therapeutic combinations (e.g., ADT + CT or hormone therapy combinations) in AR-positive HER2-negative SDC patients may be justified. As an example, a clinical case of metastatic SDC responding to three-weekly carboplatin + paclitaxel in association with combined ADT (bicalutamide 50 mg daily + leuprolide 45 mg every 6 months) was reported in 2020 [14].

Mimicking the experience gained in prostate cancer management [15], the association of a LHRH-analog, three-weekly docetaxel (75 mg/m$^2$), and abiraterone 1 g once a day may be seen as a promising approach to be explored in R/M SDC patients as well.

### *AR-Positive HER2-Positive SDC*

In the context of AR and HER2 interplay, in breast cancer, AR was shown to play an essential role in promoting the growth of HER2+ disease by a functionally significant crosstalk with the HER2 signaling [16]. Given that HER2 is more frequently amplified in high-grade SGCs (42% in R/M disease [7]), which may also bear AR overexpression, there is a compelling need to understand the best therapeutic approach for treating patients affected by these rare cancers, for which several therapies (i.e., anti-HER2 agents, ADT, CT) are available in the R/M setting.

In the cited Italian retrospective study on ADT in R/M SGCs, data about the HER2 status was available in 11 cases. In the three HER2-positive (IHC 3+) cases (all adenocarcinoma NOS, none SDC), the best objective response to ADT was a partial response in two subjects and disease progression in one patient [8]. In the ADT-resistant setting, in the only two cases with HER2 overexpression in the abiraterone phase II study, the best objective response to hormone therapy was a partial response in one subject and disease progression in the other one [10].

Emerging data have shown the activity of targeted agents in HER2-positive SGCs [17], notably trastuzumab plus CT (57 SDC, ORR 70.2%) [18], trastuzumab-emtansine ([TDM1], in 36 patients with HER2-positive cancers excluding breast and gastric/gastroesophageal junction adenocarcinomas, the only 2 responses were observed in SGC patients [67%], none SDC; in a cohort of 10 AR-positive SGC patients [SDC prevalence unknown] ORR was 90%) [19, 20], and trastuzumab-deruxtecan (ORR in a pooled analysis of two phase I studies was 58.8%; 17 HER2-positive SGC, 9 with SDC) [21].

Recent retrospective data showed that a first-line anti-HER2 approach provides a higher clinical benefit (higher response rates and longer survival) than ADT in HER2-positive AR-positive SDC patients [22]. Therefore, it is advisable to consider an anti-HER2 treatment as first-line therapeutic strategy for double positive diseased. In this context, we may hypothesize that the HER2-mediated pathway plays a stronger driving role in SDC carcinogenesis and progression than the androgen-mediated one. This observation, obtained by analyzing a vast multicenter cohort of 323 patients collected at seven Japanese institutions, would not justify a randomized study assessing first-line anti-HER2 vs ADT. Moreover, the worse outcomes observed in HER2-positive AR-positive SGC patients vs. HER2-negative AR-positive ones justify the need for an escalated approach to reach the maximum clinical benefit we may achieve with the currently available therapeutic approaches.

On the other hand, the same Japanese study found that time to second progression and duration of response in second-line treatment are similar independent of the type of treatment (anti-HER2 or ADT). Therefore, upfront ADT may be considered as a valid alternative for AR-positive HER2-positive SDC patients with comorbidities, poor performance status or older age, which may hamper the feasibility of an anti-HER2 treatment [22].

Data on anti-HER2 with ADT in SDC patients is currently lacking. Nevertheless, indirect evidence from the available literature does not pose significant concerns in terms of safety for the proposed combination. Indeed, no safety concerns were observed associating antiandrogen therapy (enzalutamide) with trastuzumab in AR-positive HER2-positive breast cancer patients [23]. Moreover, randomized clinical trials did not show an increased toxicity profile with antiandrogens like darolutamide vs. placebo in association with ADT (LHRH agonist or antagonist, such as leuprorelin, goserelin, triptorelin) and CT (docetaxel 75 mg/m$^2$ every 21 days) in prostate cancer patients [12]. These findings may justify the exploration of similar combinations in AR-positive HER2-positive SDC patients as well, to be assessed in controlled settings such as clinical trials.

As there is a highly unmet need to improve oncologic outcomes of patients with R/M AR-positive HER2-positive SGC, a possible strategy is delivering ADT with concomitant anti-HER2 treatments, with or without CT.

## *AR-Negative SDC*

AR-negative SDCs are very rare, and a second look at the diagnosis by an expert pathologist should be considered [24]. If HER2 overexpression (i.e., AR-negative HER2-positive) is found, an anti-HER2 treatment should be administered. In the rare case of double negative SDC (i.e., AR-negative and HER2-negative), molecular characterization should be performed to explore any druggable molecular alterations that may suggest off-label agents (e.g., BRAF and MEK inhibitors for BRAF V600 mutations, immunotherapy for high TMB or MSI-H etc.). Double negative R/M SDC patients might be offered systemic CT independently of molecular targets.

## Conclusion

AR overexpression is almost universal in SDCs. From a therapeutic point of view, intense staining and an expression ≥70% are the conditions to predict the clinical benefit of an antiandrogen treatment. On the contrary, AR-negative SDCs are very rare, and this diagnosis should be regarded with skepticism [24]. The following table reports the recommended treatments for R/M SDC patients based on the possible combinations of AR and HER2 expression:

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