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Trends in Surgical Research in Head and Neck Cancer

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Opinion statement

The task of surgical research is to improve the efficacy of available surgical therapeutic modalities, develop new ones, and balance this well with favorable functional outcome. Therefore, surgical research is composed of a translational and a clinical component. In translational surgical research, animal models are used to better understand the biology of head and neck cancers, but even more importantly, the biology of changes to the disease and the microenvironment created by surgical interventions. Animal models additionally allow for the development of image-guided surgery systems, novel strategies of intraoperative adjuvant treatment, and patient "avatars" to test innovative anticancer drug combinations. In clinical surgical research, surgical techniques are validated in clinical trials for effectiveness of tumor control and improvement of functional recovery of the patient. In conclusion, surgical research for head and neck cancer is an active field spanning across the entire breadth of basic and clinical science devoted to a better understanding of what surgery does to the disease and to the patient.

Introduction

Surgery for head and neck cancers remains an integral part of multidisciplinary treatments demanding from the modern surgeon not only technical skills but also increasing specific knowledge of the biology of the disease he is treating. Today, head and neck cancer surgeons team up with pathologists, radiologists, radiation and medical oncologists, and other medical specialists to offer patients a multimodality therapy that is not only efficient in terms of tumor control but also avoids functional problems after the treatment. The intrinsic difficulties to treat head and neck cancer are related to the anatomic location, remarkably complex biology, and aggressive tumor phenotype, especially when disease recurs and is becoming treatment-refractory. Despite all current advances in treatment modalities, head and neck cancer remains a mutilating disease with a significant death rate due to treatment failures. There is therefore a pressing need for *clinical and translational*

research to advance treatment technologies and tools, combining high therapeutic efficiency with beneficial functional outcome.

Surgical research in head and neck cancer thus aims at understanding the biological effects of surgery on the disease *and* its microenvironment, tries to unravel the biology of disease recurrences and treatment failures after surgery, attempts to validate existing and novel surgical treatments in clinical trials, and develops surgical strategies that improve functional recovery. Preclinical surgical animal models of human head and neck cancer help herein to understand various aspects of the biology of the disease and how surgery changes this biology, whereas clinical trials help to validate surgery as a treatment modality for clinical practice.

Pre-clinical surgical animal models

Animal models are generally adopted by the research community as invaluable tools to address a wide range of critical challenges of head and neck cancer biology and treatment. Despite substantial interspecies differences, HNSCC tumor-bearing mice, rats, hamsters, and rabbits serve today as indispensable pre-clinical models of human head and neck cancer that pave the way for novel technologies and treatments, where surgery is the central part of therapy. The most comprehensively studied and widely used models in cancer research are mice models ranging from genetically or chemically induced cancers to models that are based on the implantation of cultured HNSCC cells or fresh tumor biopsies into either immunodeficient (xenograft and patient-derived xenograft (PDX) models) or immunocompetent mice (allograft and syngeneic models) [1, 2]. The implantation of tumor cells is typically done at an orthotopic site such as the submental region [3•], anterior tongue [4], inner cheek [5•], or at a non-orthotopic site, for example for HNSCC PDX mice models [6••, 7, 8]. After microsurgical dissection of primary tumors, animals are followed for the detection of recurrences and metastatic disease to the regional lymph nodes and distant organs. Animal models are pivotal today to explore the biological background and efficacy of innovative combination therapies aimed to complement surgery, i.e., eliminating residual tumor cells after surgery while sparing the adjacent healthy tissues and structures. Surgical animal models are appropriate tools to mimic human minimal residual disease (MRD), which helps to understand mechanisms driving tumor cell spread, dormancy, immune escape, and resistance to chemo-, radio-, and molecularly targeted therapies.

Surgical mouse models to explore the biology of locally disseminating cells and residual disease

True local recurrences as opposed to second primaries are anticipated to originate from a low number of residual tumor cells resting outside of the surgical field and exhibiting prominent resistance to local and systemic therapies. Since the nature of residual cells remains largely elusive, there is a high demand for orthotopic HNSCC mouse models to mimic human postsurgical MRD. This is to disclose the molecular profile of such residual tumor cells. For this purpose, both human and mouse HNSCC cell lines have to be generated. While a large collection of human HNSCC cell lines has been established over the years [9–11], mouse cell lines, i.e., derived

from HNSCC specimens or established by in vitro transformation of oral keratinocytes, are still rare [12–14]. Given the large interest these days in the pre-clinical testing of immunotherapy, syngeneic and orthotopic models are of greatest interest.

Recent research revealed a remarkable intra-tumoral clonal heterogeneity within HNSCC tumors and associated high genetic diversification with poor outcome [15]. Clonal diversity can provide an advantage to a population of HNSCC cells benefiting from the heterogeneous tumor milieu and exploiting diversity of regulatory factors for acquiring a tissue context-specific invasive phenotype. To investigate this clonal diversity in human disease, orthotopic HNSCC models will start making use of tracing disseminating cells by tagging all the cells of the primary tumor cell population with unique genetic barcodes, a high-resolution technique utilized today for clonal tracing and dynamics [16].

In our view, many tumor cells are able to acquire an invasive phenotype and spread locally via diverse mechanisms [17]; however, only few tumor clones, which are coined recurrence-initiating cells or RICs, will be the fittest to survive at their final destination, known as niches, and to initiate recurrent disease. RICs are related to cancer stem cells (CSC) [18, 19]; however in contrast to CSCs, they would be expected to carry certain pre-existing, recurrence-promoting (epi) genetic variations or acquire *de-novo* mutations that favor the communication with the niche cells. RICs in their local niches, in particular in the perivascular space [20], benefit from paracrine and cell-cell interactions. Depending on the type of niche (i.e., peritumoral lymphatics, perineural spaces, adipose and connective tissues, muscle, and bone), different tumor-promoting cells will be recruited. Such a niche can be inhabited by a variety of cells including endothelial cells, cancer-associated fibroblasts (CAFs), and diverse types of immune cells [21]. Surgical, orthotopic animal models are operational to explore the biology of RICs in the context of wound healing-induced inflammation and immune responses and help to identify targetable mechanisms playing a key role in the development of recurrences.

Imaging and therapy of MRD in surgical animal models

Positive margins after surgical interventions are correlated with local recurrences and poor survival [22–24]. One way to improve resection techniques and therefore to avoid positive margins is to exploit the use of modern imaging techniques. Extensive work with animal models has contributed to the detection of techniques and reagents for near-infrared (NIR) fluorescent-guided surgery, which is already translated into surgical practice of head and neck oncology [25•]. Perioperative injection of NIR tracer molecules or antibody-NIR conjugates for non-invasive imaging has the intention to improve the delineation of tumor boundaries during surgery, thus, preventing margins contaminated with residual tumor cells and reducing the risk of recurrence. In this context, cell surface molecules abundantly expressed by tumor cells and by non-tumor cells from the stromal compartment of the tumor are suitable targets to specifically conceive the

tumor boundaries by real-time imaging and to detect tumor cells outside of the initial surgical margin. Antibodies that specifically recognize the epidermal growth factor receptor (EGFR), a transmembrane protein overexpressed by HNSCC cells [26], can be used with this respect. In particular, anti-EGFR 7D12 nanobody [27] and panitumumab, a fully humanized anti-EGFR mAb [28•], both conjugated to a NIR fluorophore, were investigated for their suitability as imaging reagents in surgical xenograft mice models of tongue cancer. Consequently, cetuximab-IRDye800 reagent was successfully evaluated for intraoperative detection of tumor cells in patients with HNSCC and for pathologic inspection of the freshly processed tissue sections in a phase I clinical study [29••, 30••]. As alternative to the antibody-based approach, NIR fluorescent peptide-based probes are currently tested for specific binding to tumor cell surface molecules and to cellular components of tumor microenvironment, especially tumor-associated vasculature. The integrin family of heterodimeric cell surface receptors, which are involved in the diverse signal transduction activities within the specific context of cell-cell and cell-extracellular matrix interactions, are promising targets for therapy, drug delivery, and imaging. For instance, the NIR fluorescent probe for specific integrin targeting with high capacity for tumor accumulation due to binding to $\alpha_v\beta_3$ integrin expressed by tumor and endothelial cells is worth mentioning [5•]. This reagent helped to guide resections of residual tumor pieces leading to an improvement of the recurrence-free survival rate by 50% in an orthotopic mouse HNSCC model [5•]. Conceivably, this as well as other NIR fluorescent reagents are promising tools for transoral, fluorescence-guided surgery and for concurrent intraoperative delivery of tumor cell-killing compounds.

Current efforts to combine intraoperative detection of tumor cells with their killing are illustrated by the application of ultra-small porphyrin lipoprotein-mimicking nanoparticles (PLP) featured by modalities such as positron emission tomography (PET), NIR fluorescence imaging, and photodynamic therapy (PDT) [31•]. PDT exerts its phototoxic activity by light-activated drugs in conjunction with singlet oxygen and is a fast expanding field of research with a high potential for treatment of preneoplastic head and neck lesions and cancer [32]. In particular, in a clinically relevant rabbit model of buccal SCC, multimodal PLPs demonstrated a high capability for accurate, real-time detection of primary and metastatic tumor cells and a strong curative effect after tumor bed irradiation with laser light [31•]. Although further pre-clinical studies using orthotopic HNSCC models are necessary to substantiate the targeting specificity of nanoparticle-based intraoperative treatment and to optimize their routes of delivery, PDT seems to be an attractive option for specific targeting of locally disseminated tumor cells. In clinical practice, phase I trials performed with HNSCC patients intraoperatively treated with PDT indicate safety of this therapy, although some precautions due to side effects and cardiovascular comorbities must be considered [33, 34]. These encouraging reports suggest further clinical investigations of curative efficiency of the photoactive, cytotoxic nanoparticles as relapse-preventing intraoperative therapy. Such PDT-based therapy could eventually have a great value in prevention of second field and second primary tumors (SFT and SPT)

originating from genetically abnormal tumor-adjacent mucosal areas, known as field cancerization [19], and endow surgeons with more efficient tools for local control.

Surgical mouse models to test novel treatment modalities

The translation of drug response studies from xenografted tumor cell lines is to some degree hampered by the artificial in vitro evolution of the clonal composition and by the failure to reproduce a natural microenvironment, especially upon implantation into the non-orthotopic sites. The direct transfer of patient tumor samples as a PDX into a recipient mouse is an option to overcome these shortcomings conjoined with the ability to perform therapy trials. The HNSCC PDX models are valuable tools for preclinical tests of novel drugs [35]. However, a bias in engraftment success towards HPV-negative [35] and less differentiated tumors [36] needs to be addressed in the future to represent homogeneously all HNSCC entities. Other concerns with PDX models consist in the unavoidable substitution of the original human stromal compartment by a mouse immune-deficient microenvironment. Nevertheless, PDX models are widely recognized today as a more favorable approximation of human cancer than offered by other animal models. HNSCC PDX collections and personal "avatars" hold the promise to advance personalized therapy and serve today to facilitate preclinical evaluation of novel drugs with the ability to target the most aggressive HNSCC subtypes or to eliminate the most critical cell populations for tumor recurrence pre- or postoperatively. In this respect, patients with adenoid cystic carcinoma, an aggressive salivary gland tumor, which frequently presents with perineural invasion, could potentially profit from the apoptosis-inducing BH3-mimetic molecule BM-1197. This drug has been successfully evaluated in a PDX model of adenoid cystic carcinoma as single agent that significantly delayed postsurgical recurrence [8]. The strength of PDX models is to better represent the original tumor cell heterogeneity, endowing them with a great value for the development of recurrence-preventive therapies targeting cancer stem cells. Recent studies further corroborate the utility of PDX models, i.e., by demonstrating that an adjuvant application of a humanized anti-IL-6 antibody (MEDI5117) [7] and an antibody-drug conjugate (MEDI0641) targeted to oncofetal antigen 5 T4, a N-glycosylated transmembrane protein expressed by HNSCC cells, prevented postsurgical recurrences in a HNSCC PDX model by lowering the CSC abundance and thus depleting the pool of cells with a capacity to initiate recurrent tumors [6••]. The future development of surgical HNSCC PDX models will follow the recent progress in the generation of humanized mice, which allow for the reconstitution of a functional human immune system and stromal compartment by injection of human hematopoietic stem and progenitor cells (HSPCs) into sublethally irradiated NOD/SCID/IL2rg$^{-/-}$ (NSG) mice to better mimic a human microenvironment [37••].

Organ transplantation, regeneration, and digital innovations

Organ transplantation is an important field of development in surgical research. To be able to replace diseased organs of the head and

neck by allografts or regenerated organs is of major interest and defines an important domain of current activity. In the head and neck region allo-transplantations of the larynx, laryngo-trachea, tongue, and face have been done. While few transplants of the larynx have been performed with the first in 1998 in the Cleveland clinic [38], a subsequent one in the USA and a series in Colombia, many more face transplants have been done (>27) [39]. A study reviewing the outcomes of face transplants demonstrated good sensory re-innervation and some recovery of thermal and mechanical sensation. Full restoration of sensation was seen by approximately 8 months. There was however poorer motor recovery. Authors reported a significant improvement in the ability to smell, eat, smile, and speak. Pain also seemed to be reduced. All of this was associated with a significant improvement of quality-of-life and reduction of anxiety and depression [40, 41]. Finally, one tongue allo-transplantation has been performed in Austria [39].

The difficulties inherently associated with allotransplanted organs in the head and neck region are the requirement for immunosuppression, which poses problems in patients treated for cancers. However, novel strategies limiting the degree of immunosuppression while preventing sufficiently acute or chronic rejection of the donor organ combined with antitumor activity are under investigation. Low-dose everolimus, an mTOR inhibitor with proven activity against squamous cell carcinomas, successfully prevented laryngeal allograft rejection 60 days post transplantation in a mouse model [42]. In other experiments, a combination regimen of everolimus with an antibody against the αβ-T-cell receptor (TCR) induced graft tolerance for 10 months with a total of 15 days of immunosuppression only [43]. Lastly, the use of modified immature dendritic cells prior to transplantation conferred donor-specific tolerance for up to 60 days [44].

The use of regenerated organs or organ parts in the head and neck region is also of great interest. These organs or organ parts typically consist of a biological scaffold or an allotransplant that is seeded with patient-derived cells. Advances have been made with respect to the trachea and the laryngo-tracheal complex. The first transplant of fully engineered tissue in the head and neck region was performed in 2008 and consisted of a replacement of the left main bronchus by a biological scaffold seeded with patient-derived epithelial and cartilage cells. Four months out from the procedure biopsies demonstrated revascularization and full tissue integration [45]. Since then, concepts were pursued using allogeneic trachea wrapped with recipient-derived free flaps [46] or synthetic scaffolds seeded with autologous stem cells [47]. For laryngeal reconstruction, concepts are under investigation using de-cellularized human larynges later seeded with recipient-derived cells. This concept seems promising given the low antigenicity of the donor organ after de-cellularization [48].

Digital innovations are another area of interest in surgical research. In particular, 3D navigation systems based on imaging, i.e., CT, MRI, and PET, allow for precision biopsies, tumor resection with adequate margins, and adequate reconstruction. In particular for mandibular

and mid-face bony reconstructions, these techniques are already in use and will be further developed in the future [49].

Surgical trials and associated challenges

Clinical surgical research on head and neck cancer aims at translating data obtained from translational research into clinical trials [50]. Currently, a fifth of surgical trials in general are abandoned, less than half published, and surgical trials are currently recruiting only 5% of funding in oncology [51]. Among 473 US trials registered at the ClinicalTrials.gov between 1996 and 2014 surgery was found as an underrepresented arm of therapy [52]. This delineates the magnitude of the problem. Thus, clinical researchers have to face that surgical experimental treatments are difficult to define, have components that are not foreseen, that surgical experimental treatments are multi-layered and thus less standardized. The challenges are therefore the standardization of experimental surgical treatments, quality assurance prior, during, and after the trial, to overcome randomization issues, to prepare a professional framework for the conduction of the trial, to avoid trials with treatments that do not reflect reality, and funding.

Surgical trials have various purposes. One is to evaluate novel surgical treatment strategies in terms of oncological outcome, but also in terms of functional outcome and compare this with current standard of care, which may be a non-surgical treatment. Other aims of surgical trials are the implementations of state-of-the-art surgical techniques. This can be compared to phase 1 trials, in which novel drugs are tested for toxicity. However, surgical phase 1 studies are rather prospective cohorts of patients treated with the new surgical strategy and rigorously assed for peri- and postoperative complications. Finally, surgical trials can help to characterize drug effects in newly treated cancer patients in so-called "windows-of-opportunity" trials, in which patients are scheduled for a surgical resection and the window prior treatment is used as an opportunity to challenge the tumor with a novel drug. The data obtained from the analysis of such tumors provides valuable information about early effects in tumors that are yet treatment-naive.

Windows-of-opportunity trials in head and neck cancer

Windows-of-opportunity trials for head and neck cancer have inherent difficulties that are referring to pathological efficacy endpoints that do not yet exist in a reliable way [53]. However, various drugs and drug combinations have been investigated in such trials. An interesting drug target is the EGFR, being overexpressed in more than 90% of HNSCCs [26]. Various windows-of-opportunity trials have thus been conducted with either EGFR antibodies (Cetuximab [54]) or EGFR and pan-HER kinase inhibitors (i.e., Erlotinib [55], Lapatinib [56], Dasatinib [53], Afatinib (NCT01415674 and NCT01538381), Dacomitinib [53]), and even non-selective COX-inhibitors (i.e., Sulindac [57]) were used. Tumor shrinkage, apoptotic index, and KI67-expression were typically used as primary endpoints. However, the best performing endpoint for efficacy seems to be ^{18}FDG-PET, because a strong correlation between delta

maximal standardized uptake values (ΔSUV_{max}) and residual tumor cellularity in resected specimens was found [54].

Trials to investigate surgery as a function-preserving treatment modality

Surgery for head and neck cancer has evolved in various directions, such as the development of reconstructive surgery [58•, 59•] and the introduction of micro-vascular free flaps [60•]. Also, novel endoscopic surgical approaches, such as transoral laser microsurgery (TLM) and transoral robotic surgery (TORS) were developed for oropharyngeal and laryngeal SCCs resection [61•, 62–64]. All these developments have in common to improve the functional outcome and decrease operative time in selected cases. While there are currently no surgical trials on evaluating free flap surgery in terms of functional benefit for the patient, several trials are currently conducted or in preparation that assess the potential benefit of transoral surgery (TOS).

The "Best-of" trial

The "Best-of" trial is a phase III randomized controlled trial comparing intensity-modified radiation therapy (IMRT) with TOS in early stage oropharyngeal cancers. The trial considers the development of new technology in the field of radiation therapy, i.e., IMRT, and surgery, i.e., TOS, with the potential of a better functional recovery as a consequence of a more targeted therapy with less access trauma and collateral damage. The primary endpoint in "Best-of" is the evolution of swallowing recovery within 1 year after randomization and will thus capture the entire swallowing situation and recovery including the period of treatment [65••].

The PATHOS trial

This trial is a phase II randomized controlled trial looking at the effects of de-escalating adjuvant therapy after TOS in operable HPV-positive oropharyngeal cancers. Patients are distinguished into histological risk-groups and intermediate-risk patients (T3, N2a or N2b, perineural invasion, vascular invasion, close margins (1–5 mm)) are randomized into a standard arm with 60 Gy of adjuvant RT versus an experimental arm of 50 Gy. The high-risk group (positive margins (<1 mm) with negative marginal biopsies and/or extra-capsular spread (ECS)) is randomized into a standard arm with Chemo-RT (60 Gy) versus an experimental arm with RT (60 Gy) only. The de-escalation is thought to help with a faster and better recovery of swallowing, and this is why the primary endpoint in this trial is swallowing function 1 year after finishing treatment [66].

The ORATOR trial

This phase II randomized trial compares TORS with RT or chemo-RT for patients with HPV-positive operable oropharyngeal cancers up to an N2b stage. However, patients with signs of ECS on imaging are excluded. The

primary endpoint is swallowing recovery at 1 year after finishing treatment [67].

ECOG 3311

This trial is a phase II trial with the aim of de-escalating adjuvant treatment similar to the PATHOS trial. Again, patients with operable oropharyngeal cancers are treated in this trial and categorized into risk-groups after TORS. The intermediate-risk group (close (<3 mm) margins, <1 mm ECS, 2–4 metastatic lymph nodes)) is randomized into a standard arm of 60 Gy postoperative radiation and an experimental arm of 50 Gy. The high-risk group (>1 mm ECS or >5 metastatic lymph nodes, positive margins) as opposed to the PATHOS trial is not object to a de-escalation. The endpoint is primarily progression-free survival at 2 years. Secondary endpoints include health-related quality-of-life and swallowing recovery [65••].

Surgical trials combining surgery with novel agents

Remarkable results in the treatment of head and neck cancer patients in particular in the metastatic and recurrent situation have been achieved with the introduction of immunotherapy, in particular with the introduction of checkpoint blockade. In CheckMate 141 Nivolumab, a PD-1-inhibitor was demonstrated to provide an overall survival of 36 versus 16.6% at 1 year in recurrent/metastatic HNSCCs [68]. It is therefore logical to test the effect of these novel agents in the context of surgically treated HNSCC patients. Therefore, in a currently recruiting trial (NCT02296684) at Washington University in St. Louis and Dana Farber Cancer Institute, operable HNSCC patients are treated neo-adjuvantely and adjuvantely with Pembrolizumab, a PD-L1-inhibitor. Pembrolizumab is only given after finishing (chemo)-RT in the case of high-risk features, i.e., ECS or positive margins.
The future focus of clinical studies will be the identification of biomarkers [69] helping to predict benefits or harms of therapies. The long-term goal is evidence-based counseling of patients to offer a personalized therapy, where upfront surgery could be an integral part of treatment. Predictive markers should help to foresee what functional outcome is to be anticipated based on each treatment option and consequently help to select patients who will benefit from one or the other therapy.

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Compliance with Ethical Standards

Conflict of Interest
The authors declare they have no conflict of interest.

Human and Animal Rights and Informed Consent
Although this article refers to previously conducted studies with human and/or animal subjects performed by the authors, no new studies were conducted for this particular article.

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