4th INTERNATIONAL SYMPOSIUM ON TUMOR-HOST INTERACTION IN HEAD AND NECK CANCER

in conjunction with the
1st AI IN HEAD AND NECK ONCOLOGY – SYMPOSIUM

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ABSTRACTS

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Working Group **Oncology** of the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery

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In-depth Characterization of the AREG-EGFR Pathway in HNSCC

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Objective
Advanced head and neck squamous cell carcinoma (HNSCC) presents a significant clinical challenge due to low response rates to current therapies and a lack of predictive biomarkers. Previously, EGFR activity subtypes were identified, being characterized by a positive correlation between EGFR activity, epithelial-mesenchymal transition (EMT) levels, and the expression of EGFR ligands, particularly AREG. Since EMT promotes therapy resistance and metastasis, this study aims to unravel the molecular mechanisms of AREG-EGFR mediated EMT in HNSCC invasion.

Methods
A publicly available HNSCC scRNA-seq dataset GSE181919 was utilized to investigate this process. Cells were clustered based on AREG expression, EMT score, and EGFR activity. AREG targets and regulators were identified by comparing differential gene expression and protein activity of these clusters and the gene regulatory network of EGFR-ACTIVITY subtypes.

Results
AREG expression exhibited the highest correlation with EMT scores among all EGFR ligands and demonstrated the strongest receptor-ligand interaction between cell types. Analysis of scRNA-seq clusters revealed that AREG-expressing cells are both EMT-positive and highly proliferative, showing enrichment for MAPK, WNT, and PI3K signaling pathways. Conversely, AREG-negative cells displaying high EMT/EGFR activity were characterized by activation of pathways such as hypoxia and TRAIL, alongside a low proliferation rate. FOSL1 was identified as a transcriptional regulator and a direct target of AREG.

Conclusion
This study identifies a distinct AREG-driven EMT phenotype in HNSCC where the highly proliferative, AREG-expressing cells are suggested to be critical drivers of tumor metastatic spread. FOSL1 is highlighted as a mediator in this process, representing a potential novel biomarker and a therapeutic target for HNSCC.

Defining Predictive Immune Signatures by Immune Monitoring and Machine Learning from the Blood of HNSCC Patients Receiving Postoperative Radio-(chemo)therapy in the Prospective DIREKHT Trial

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Immunological biomarkers are becoming increasingly important in the staging and treatment of various solid tumors. However, in HNSCC, limited research has been conducted on blood-based biomarkers. To address this gap, the prospective DIREKHT study (NCT02528955) incorporated a translational research program focused on longitudinal monitoring of patients" immune profiles throughout treatment. The goal was to identify predictive immune signatures associated with prognosis and response in HNSCC.

The immune status of 70 HNSCC patients was assessed before and after R(C)T, as well as during follow-up. Using a flow cytometry-based assay, 45 immune parameters were analyzed from whole blood samples. These data were used to train a machine learning model to predict disease-free survival (DFS). The model was built through a data-driven variable selection process followed by logistic regression, and validated using nested stratified K-fold cross-validation with repeated sampling.

A predictive immune signature comprising 29 parameters was identified, with HLA-DR$^+$ T cells, HLA-DR$^+$ monocytes, and basophils emerging as key contributors. The model achieved a Matthews correlation coefficient (MCC) of 0.681 and was equally informed by pre- and post-treatment immune profiles, highlighting the importance of tracking immune dynamics throughout R(C)T. Both adaptive and innate immune parameters were included in the signature. Adding clinical variables did not enhance model performance (MCC = 0.678); however, it yielded a similarly effective model that combined post-treatment immune markers with clinical characteristics.

This study underscores the relevance of immune profiling in HNSCC for the discovery of prognostic biomarkers. The identified immune signature shows promise in predicting DFS in patients undergoing R(C)T and may support future strategies for personalized treatment. Further validation in larger cohorts is necessary for translation into clinical practice.

Personalized Neoantigen Screening and Precision Immunotherapy for Head and Neck Squamous Cell Carcinoma Based on Multi-omics and Organoid-on-a-Chip Technology

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Background
Neoantigen peptides are valid targets for individualized immunotherapy in head and neck squamous cell carcinoma (HNSCC). However, the conventional peptide-selection workflow lacks assessment within the native tumour microenvironment, resulting in low clinical response rates. This study aimed to establish an end-to-end pipeline encompassing peptide-library construction, algorithmic prioritisation and organoid-on-chip validation to identify dominant peptides that potently activate $CD8^+$ T cells within authentic tumour surroundings.

Methods
Whole-exome, transcriptome and immunopeptidome data from 249 patients were integrated to build a library of 16,000 mass-spectrometry-confirmed, HLA-presented tumour peptides. A peptide–HLA–TCR tripartite network was constructed and subjected to two-step PageRank screening (global PageRank followed by Personal PageRank). Candidate peptides were then examined by ELISPOT, tetramer staining and patient-derived xenograft (PDX) models to confirm immunogenicity. Finally, tumour organoids and autologous PBMCs were co-cultured on a microfluidic chip; impedance, apoptosis and effector-molecule release were monitored in real time to rank peptides by integrated cytotoxicity score.

Results
We constructed a library of 16,000 MS-confirmed neoantigen peptides, 74% arising from mutations or RNA editing, 18% from gene fusions/alternative splicing and 8% HPV-related. Per-patient presentable peptides were reduced from a median of 126 (pure in-silico prediction) to 38 (MS-confirmed), lowering the false-positive rate from 58% to 12%. The personalised prioritisation pipeline narrowed the candidate pool to 10 peptides per patient, increased ELISPOT positivity from 15% to 65% and yielded a PDX tumour-growth inhibition ≥50% for 65% of selected peptides. On the organoid–immune chip the top 10 peptides maintained <20% target-cell viability after 72 h.

DNA Copy Number Alteration-Status of Head and Neck Squamous Cell Carcinomas is associated with Distinct Radiomic Phenotypes

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Background
Approximately 70-90% of head and neck squamous cell carcinomas (HNSCC) exhibit extensive DNA copy number alterations (CNA-positive), whereas 10-30% have genomes with somatic driver mutations, few CNAs (CNA-negative) and longer overall survival. By quantifying tumor shape, intensity, and texture from imaging data, *Radiomic* features reflect tumor biology and prognosis. This study investigated whether CNA-subtypes of HNSCCs display distinct radiomic phenotypes.

Methods
Patients were hierarchically clustered to define CNA subtypes (based on DNA copy number analysis results from The Cancer Genome Atlas, n=522). 1037 radiomic shape, intensity, and texture features were extracted from primary tumors on pretherapeutic contrast-enhanced CT of n=114 patients. Univariate logistic regressions examined feature-CNA status-associations. CNA status classification models were built by integrating feature selection with machine learning algorithms and validated using nested cross-validation.

Results
CNA-negative HNSCCs demonstrated favorable overall survival (n=160/522, p=0.007). In univariate analysis, 190 radiomic features were significantly associated with the CNA status, of which 29 texture and intensity features remained significant after correction for multiple comparisons. An optimized support vector machine-based model predicted the CNA status with an area under the curve (95% confidence interval) of 0.71 (0.60–0.83).

Conclusions
CNA subtypes are predominantly reflected by radiomic texture and intensity features. These findings help elucidate the biological underpinnings of radiomic features in HNSCC and may support the translation of radiomics-based applications into clinical practice. As the CNA status may gain clinical relevance for prognosis, staging, and therapy, refined radiomics models could potentially facilitate image-based CNA subtyping in future precision oncology workflows.

Machine Learning–Based Prediction of Overall Survival from Early Surrogate Endpoints in Head and Neck Cancer Clinical Trials

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Question
Can overall survival (OS) be reliably estimated from early efficacy measures in head and neck cancer trials? Which surrogate measures best correlate with OS?

Methods
We systematically reviewed first-line trials (Jan 2010–Mar 2025) in recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC). Five machine learning (ML) models were evaluated to predict OS from early surrogate efficacy endpoints (objective response rate (ORR), disease control rate (DCR), median progression-free survival (mPFS), median duration of response (mDOR), and 1-year OS rate), along with patient characteristics (HPV status, ECOG, PD-L1). External validation was performed using a retrospective real-world patient dataset from Samsung Medical Center (n=298).

Results
Ninety treatment arms (26 immune checkpoint inhibitor (ICI)-based, 64 non-ICI regimens) were analyzed. The 1-year OS rate showed the strongest correlation with median OS (r=0.87, P<0.001) across regimen types. ORR and mPFS correlated with OS overall but were significant only in the non-ICI subgroup. Additionally, mDCR showed significant correlation with OS in the non-ICI cohort only. Among different ML models, the Elastic Net model achieved strong predictive performance (clinical trial held-out test set r=0.74, P<0.001; real-world stimulated validation set r=0.75, P<0.001). Among patient characteristics, ECOG and HPV positivity consistently influenced OS predictions, while PD-L1 status impacted OS only in ICI-based regimens.

Conclusions
The Elastic Net model effectively links surrogate efficacy endpoints with OS, with the 1-year OS rate and ORR showing the strongest correlation. This ML-based approach supports interpretation of early trial results and prediction of survival benefit in R/M HNSCC clinical trials.

Fig. 1

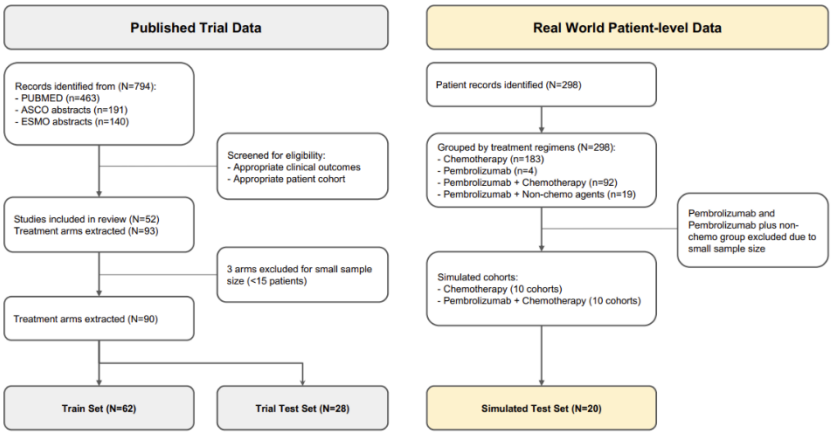


Fig. 2

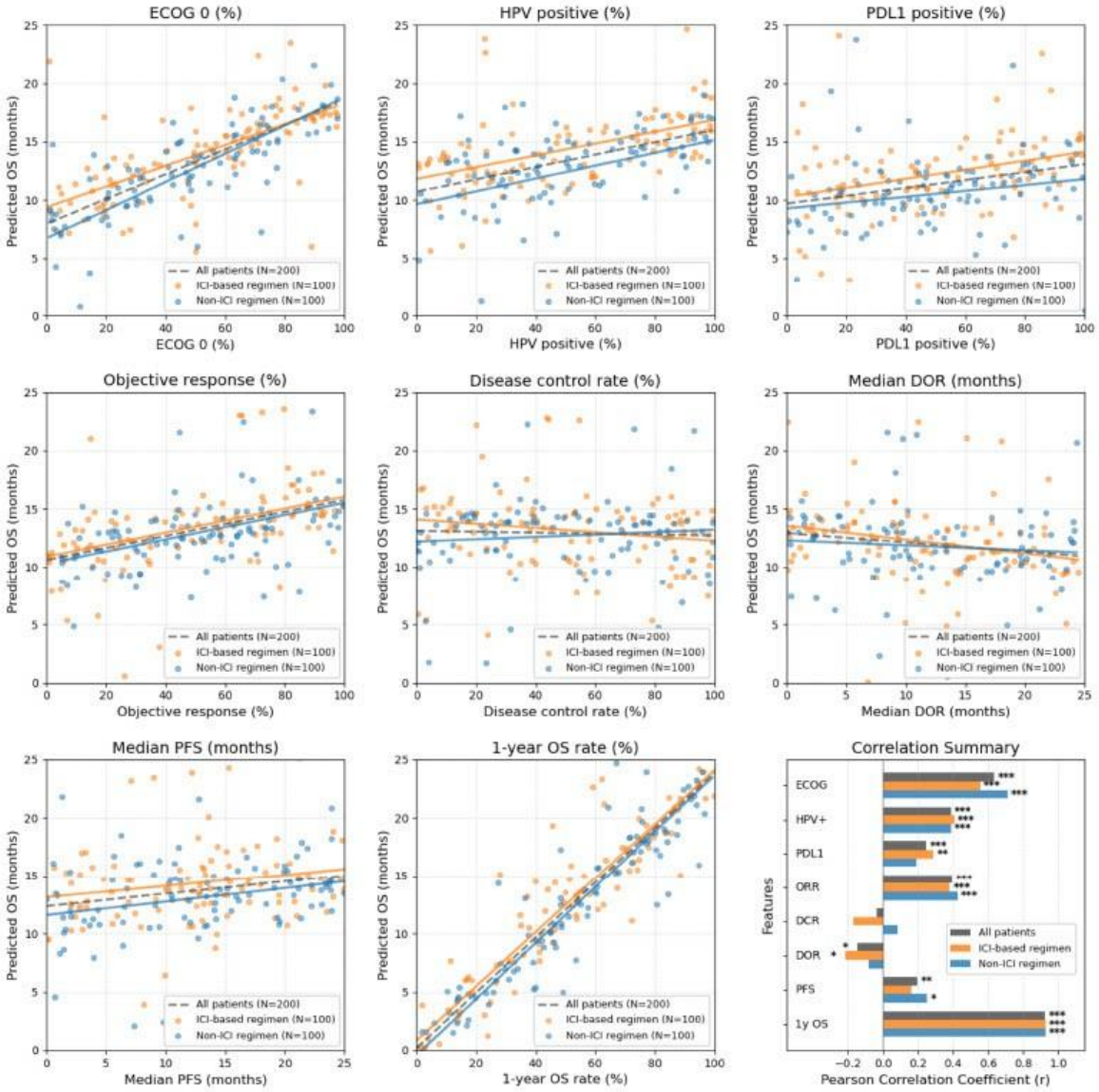
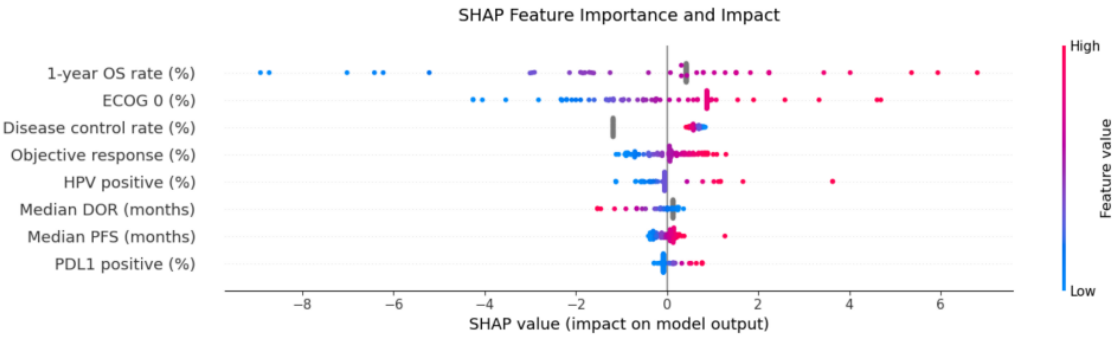

ECOG 0 (%)
HPV positive (%)
PDL1 positive (%)
Objective response (%)
Disease control rate (%)
Median DOR (months)
Median PFS (months)
1-year OS rate (%)
Correlation Summary
Predicted OS (months)
All patients (N=200)
ICI-based regimen (N=100)
Non-ICI regimen (N=100)
Features
ECOG
HPV+
PDL1
ORR
DCR
DOR
PFS
1y OS
Pearson Correlation Coefficient (r)

Fig. 3


SHAP Feature Importance and Impact
1-year OS rate (%)
ECOG 0 (%)
Disease control rate (%)
Objective response (%)
HPV positive (%)
Median DOR (months)
Median PFS (months)
PDL1 positive (%)
SHAP value (impact on model output)
High
Feature value
Low

Vision-Weighted Diagnostic Gain (VWDG) in the Diagnosis of Oral Malignant Lesions: A Multimodal Comparative Analysis

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Background
Early diagnosis of oral malignant lesions is critical for improving outcomes in head and neck oncology, yet overlapping clinical features make recognition difficult. Multimodal large language models (LLMs), which combine text and image inputs, offer new diagnostic opportunities. We applied vision-weighted diagnostic gain (VWDG), a novel metric to quantify a model"s ability to use visual input to correct diagnostic errors, in a comparative evaluation of ChatGPT-5 and Gemini 2.5 on real clinical cases.

Methods
We analyzed 150 anonymized patient vignettes with structured text and intraoral photographs. Each model was tested under text-only and multimodal (text + image) conditions. Diagnostic accuracy was measured at Top-1, Top-3, and Top-5 ranks against expert consensus. VWDG was calculated as absolute gain, relative gain, and error reduction. Regression adjusted for case difficulty.

Results
ChatGPT-5 showed substantial vision-driven improvements. At Top-1, accuracy rose from 27.3% (text) to 50.0% (multimodal) (+22.7 pp; OR 3.77, $p < 0.0001$). At Top-3, accuracy increased from 49.3% to 78.7% (+29.3 pp; OR 4.45, $p < 0.0001$), halving errors. At Top-5, performance rose from 77.3% to 92.0% (+14.7 pp; OR 3.10, $p = 0.003$). In contrast, Gemini 2.5 showed minimal or negative vision gains: Top-1 (62.0%→66.0%, +3.3 pp; OR 1.14, ns); Top-3 (76.7%→80.0%, +3.3 pp; OR 1.08, ns); Top-5 (90.7%→85.3%, –5.3 pp; OR 0.92, ns).

Conclusion
On real clinical cases, ChatGPT-5 achieved robust VWDG with significant error reduction, while Gemini 2.5 showed negligible or adverse effects. VWDG provides a reproducible benchmark of vision capability in multimodal LLMs, underscoring ChatGPT-5"s potential as a decision-support tool for early detection in oral and head and neck cancer.

Keywords
Vision-weighted diagnostic gain, ChatGPT-5, Gemini 2.5, oral malignant lesions, multimodal AI, real clinical cases

Artificial Intelligence–Based Image Recognition for Diagnosing Oral and Oropharyngeal Cancer and Leukoplakia

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Question
Visual assessment remains a cornerstone in diagnosing squamous cell carcinoma, including oral (OSCC) and oropharyngeal (OPSCC) squamous cell carcinoma, which present with diverse clinical appearances. Early detection and continuous follow-up are essential for improving survival but remain limited by access to expert evaluation. The integration of image recognition into artificial intelligence (AI) introduces new opportunities for digital aftercare, remote triage, and population-level screening in head and neck oncology. This exploratory study evaluated the diagnostic performance of AI in distinguishing OSCC, OPSCC, leukoplakia, and non-lesion oral mucosa based on clinical photographs.

Methods
A dataset of intraoral images was analyzed, including OSCC, OPSCC, leukoplakia, and non-lesion controls. ChatGPT was prompted to provide the most likely diagnosis under three conditions: (i) image only, (ii) image plus clinical history, and (iii) clinical history only. Diagnostic performance was assessed by two independent reviewers using sensitivity, specificity, and accuracy, and summarized with a modified Artificial Intelligence Performance Index (AIPI);

Results
When image and clinical history were provided, ChatGPT achieved the highest diagnostic performance—SCC sensitivity 100%, specificity 88.2%, accuracy 91.1%, and leukoplakia accuracy 95.6%. With image only, SCC detection decreased markedly (sensitivity 18.2%, accuracy 35.6%), while leukoplakia remained accurately identified (84.4%). Using clinical history alone led to overall reduced performance (SCC accuracy 84.4%; leukoplakia 64.4%; non-lesion 57.8%);

Conclusions
AI demonstrated excellent diagnostic accuracy for leukoplakia using image recognition alone and high sensitivity for SCC when supplemented with clinical history. Although not yet suitable for autonomous diagnosis, with further refinements, AI tools could enhance early detection, aftercare, and population screening in oncology.

Assessment of Pre- and Post-Treatment Multidisciplinary Tumor Board Recommendations with a Locally Operating Large Language Model (LLAMA 3.3) in Head and Neck Cancer Management

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Objective
Large language models (LLMs) are increasingly used as tools in medical decision-making. This study evaluates the accuracy of a locally operating LLM (lama 3.3) in generating recommendations for head and neck squamosa cell carcinoma (HNSCC) compared with multidisciplinary tumor board (MDT) decisions, in accordance with European data protection regulations.

Methods
A retrospective unicenter study including 676 patients with HNSCC, resulting in 1,352 clinical scenarios. Llama 3.3 was locally optimized through systematic prompt engineering using summarized German, European, and National Comprehensive Cancer Network (NCCN) guidelines. Pre- and post-therapeutic MDT recommendations were compared with LLM outputs. Concordance was categorized as complete agreement, guideline-compliant deviation, or non-guideline-compliant deviation. Model accuracy was evaluated using the F1 score and Matthews correlation coefficient (MCC), which provide complementary measures of classification performance.

Results
Comparison of MDT and LLM recommendations revealed complete agreement in 74% of pre-therapeutic and 83% of post-therapeutic cases. Including partial concordance, the guideline compliance reached 95% and 91%, respectively. For curative versus palliative concepts, the LLM achieved 97% accuracy. Discordances were primarily due to missing ECOG, inoperability data, and occasional overestimation of curative intent in stage IVc cases with solitary metastases. Subgroup analyses confirmed high reliability across tumor sites and stages. Nevertheless deviation might occur depending on the subcategory of HNSCC.

Conclusion
Locally operating LLMs, such as Llama 3.3, can generate therapy recommendations with high concordance to MDT decisions while ensuring compliance with EU data protection regulations. Even though, MDTs remain essential for individualized care, AI-based guideline integration may streamline data synthesis, enhance standardization, and improve overall efficiency.

Deep Learning-based recognition and classification of voice disorders.

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Objective
Voice disorders, such as dysphonia, laryngitis, or paresis, are widespread. If not detected early, they may become chronic or lead to more severe symptoms. Previous studies have shown promising results in detecting pathological voices using artificial intelligence (AI). However, existing approaches in classifying into specific classes still show potential for improvement. The aim of this work was to recognize pathological voices and subsequently classify them into specific pathologies.

Methods
In this work, the Saarbrücker Voice Database was used. Established deep learning models were trained to define baselines. To enhance model performance, the training dataset was augmented with synthetic data generated with Text-to-Speech models. Furthermore, different approaches to combine models were investigated, including a learned weighting for an ensemble model. The influence of adding a sex embedding to the model was also analyzed. To evaluate model performance accuracy and F1-score were utilized. Additionally, sensitivity and specificity were calculated for the recognition task and precision and recall for the classification task.

Results
In both the recognition and classification task the ensemble with learned weighting and an additional sex embedding achieved highest performance metrics. In the recognition task, the model achieved a mean sensitivity of 0.92 and F1-score of 0.90. While the model for the classification task achieved a mean accuracy of 0.73 and F1-score of 0.66.

Conclusion
In voice disorder recognition, the model showed reliable results. This enables an objective and time-efficient early detection of voice disorders. The use of AI models thus offers a promising addition to clinical diagnostics and could be integrated into telemedical screening tools in the future.

Fundamental Mechanisms of Tumor Progression and Immunity

Understanding Early Steps of Local Invasion in HNSCC

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Objective
Head and neck squamous cell carcinomas (HNSCC) show a strong propensity to local invasion, which is mirrored in tumor budding, a high proportion of locally advanced tumors at first diagnosis, frequent local recurrences despite multi-modal therapy, and consequently poor overall prognosis. Understanding molecular aspects of early tumor cell dissemination is therefore important to improve patient treatment.

Methods
We have combined 3D-models of EGFR-mediated local invasion allowing function-based cell enrichment with RNA-sequencing (RNAseq) to study locally invasive HNSS cells. Experimental findings have been cross-referenced with real-world bulk and single-cell RNAseq to identify effector molecules and predictive markers for Cetuximab-based therapy.

Results
Upon function-based enrichment of locally invasive cells after EGFR induction, gene signatures associated the invasive phenotype have been identified. Cross-referencing with gene expression profiles of single malignant HNSCC cells specified a 16-gene signature involved in EGFR-mediated local invasion that is related to epithelial-to-mesenchymal-transition and EGFR-MAPK-activity in patients. Effector molecules ITGB4, CD73, and SPHK1 were further validated in functional assays using genetic knockouts, antagonizing antibodies, and small molecule inhibitors. A high expression of selected genes from the 16-gene signature was further correlated with substantially improved response to Cetuximab treatment in rodents and humans.

Conclusion
Understanding molecular aspects of therapeutic targets such as EGFR and others in HNSCC opens new avenues for combinatorial treatment options and response prediction.

Stromal Plasticity and Androgen Signaling Underpin Sex Differences in HNSCC

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Head and neck squamous cell carcinoma (HNSCC) is more prevalent in males than females, yet the biological basis for this disparity remains unclear. Using mice exposed to 4NQO, we found that carcinogenesis is accelerated in males and that syngeneic HNSCC cell lines grow faster in male hosts when compared to female hosts. This growth advantage was inversely associated with anti-tumor immunity, as female mice displayed stronger immune activity and delayed tumor progression. To illuminate clinically relevant cellular differences between males and females, we compared scRNA-seq datasets from human HNSCC, stratified by sex chromosome gene expression.[HJaPD1] Male tumors were enriched for inflammatory CAFs (iCAF) and immune-exhausted CD8 T cells, whereas female tumors exhibited a predominance of myofibroblastic CAFs (myCAF) with a modest rise in activated CD8 T cells. Ligand–receptor analyses suggested that in males the tumor–CAF interactome is preferentially driven by IL-1 signaling, that is known to regulate inflammation. Staining of human and murine HNSCC tumors showed that male tumors have an increased expression of prostaglandin-endoperoxide synthase 2 (COX2/PTGS2) also in tumor cells as compared to females. In male mice surgical castration delayed 4NQO-driven tumorigenesis and reduced COX2 and IL-1β expression. Pharmacologic androgen blockade with enzalutamide in tumor-bearing mice also decreased COX2 expression but increased TGF-β. Mechanistically, IL-1R1 is elevated in iCAFs relative to myCAFs, and enzalutamide or TGF-β reduced IL-1R1 expression. Furthermore, dihydrotestosterone (DHT) increased the expression of the androgen receptor, COX2, and IL-1b, as well as enhancing iCAF differentiation. *In vivo* genetic interrogation demonstrated that the male–female gap in tumor susceptibility was abrogated in IL-1β-knockout mice, whereas IL-1α-knockout mice retained sex differences, albeit with delayed tumor initiation. Collectively, our data indicate that IL-1–driven CAF plasticity and androgen signaling cooperatively shape a sex-biased TME that promotes HNSCC initiation and progression.

Neutrophil-Specific Targeting of STAT3 Impairs Tumor Progression via the Expansion of Cytotoxic CD8$^+$ T Cells in Head and Neck Cancer

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Neutrophils have emerged as critical drivers of tumor progression and are frequently linked to poor prognosis across cancers, including head and neck cancer (HNC). Despite ongoing efforts to therapeutically target neutrophil functions, clinical success remains limited. In this study, we addressed the possibility of blocking STAT3 signaling in neutrophils as a targeted therapeutic intervention in HNC. Conditional deletion of *Stat3* in a neutrophil-specific manner (*Ly6G^{cre}Stat3$^{fl/fl}$* mice) significantly impaired tumor growth and metastasis in mice. Neutrophils isolated from these mice exhibited a strong antitumoral phenotype, with increased MHCII, CD80/86 and ICAM-1 expression. Immune profiling of tumors and tumor-draining lymph nodes of these mice revealed significant enrichment of CD8$^+$ T cells (granzymeB^{hi}, perforin^{hi} and IFN-γ^{hi}) with strong cytotoxic activity. To further translate these findings to human settings, we blocked STAT3 signaling in HNC cancer patient neutrophils via the small molecule inhibitor LLL12 and assessed its effects on patient-derived tumor explants. In agreement with the in vivo mouse data, we observed the expansion and activation of cytotoxic CD8$^+$ T cells in such explants. To test the therapeutic applicability of STAT3 targeting, we utilized myeloid cell-selective STAT3 antisense oligonucleotide (CpG-STAT3ASO) to target neutrophils in vivo in tumor-bearing mice. Consistent with previous results, neutrophil-specific STAT3 knockdown impaired tumor growth and enhanced cytotoxic T cell activity in the tumors and tumor-draining lymph nodes of treated mice. These findings highlight STAT3 signaling as a deleterious pathway supporting the protumoral activity of neutrophils and suggest that neutrophil-targeted STAT3 inhibition is a promising opportunity for cancer immunotherapy, providing novel insights into targeted therapeutic avenues.

Fundamental Mechanisms of Tumor Progression and Immunity

Granulopoiesis and Thrombopoiesis Aberrantly Synergize in Cancer to Promote Tumor Progression

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Myeloid cells acquire pro-tumorigenic properties in cancer, but the underlying mechanisms remain ill-defined. Employing human materials and animal models, we identified that bone marrow megakaryocytes adopt an anabolic-inflammatory state in solid cancers including head and neck squamous cell carcinoma (HNSCC), which attracts aged neutrophils having returned from the circulation. Subsequently, these neutrophils release myeloperoxidase, which destabilizes megakaryocyte extensions (=proplatelets) through hypochlorous acid-dependent processes. This enables monocytes to shed proplatelets into the bloodstream, thus generating immature/reticulated platelets with highly immunomodulatory properties. Concomitantly, megakaryocyte interactions with neutrophils freshly produced by tumor-initiated granulopoiesis shift their phenotype pro-angiogenic, before these neutrophils leave the bone marrow. In the tumor, immature/reticulated platelets recruit pro-angiogenic neutrophils for aberrant vessel formation, collectively fueling malignant growth. Mechanistically, these events rely on excessive tumor-associated interleukin-6 and/or (subsequent) thrombopoietin production, which correlates with impaired survival in HNSCC and other solid malignancies. Interfering with this tumor-elicited synergistic dysregulation of granulo- and thrombopoiesis might represent an innovative concept to counteract cancer progression.

This study is supported by Deutsche Forschungsgemeinschaft (DFG).

Rethinking Androgen Deprivation Therapy for Salivary Duct Carcinoma: Epithelial–Mesenchymal Transition as a Potential Mechanism of Therapeutic Resistance and Disease Progression

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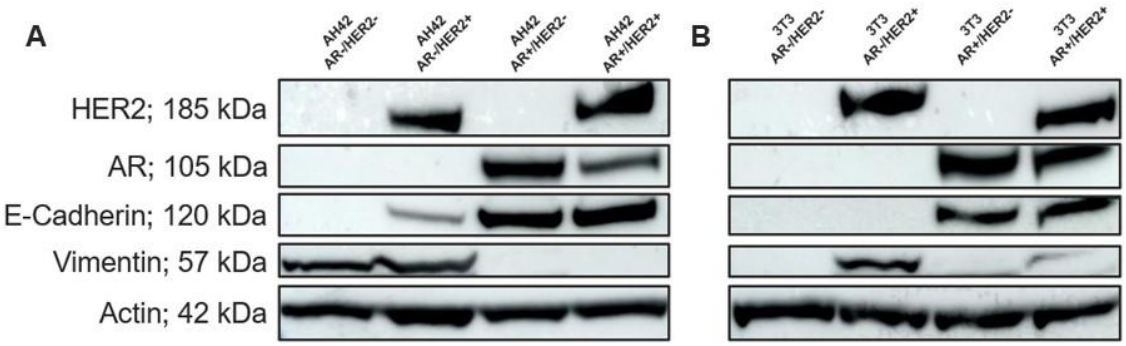
Question
Salivary duct carcinoma (SDC) accounts for up to 18% of SGC and has a 5-year survival of 40-54%. Overexpression of *androgen receptor* (AR) and *human epidermal growth factor receptor 2* (HER2) is found in >90% and 30-50%, respectively. Despite AR- and HER2-positivity guiding targeted treatment, antiandrogen therapy (AAT) has a median progression-free survival (mPFS) of less than 9 months. This is substantially shorter than prostate cancer (PCa), where mPFS is up to 54 months. Epithelial-mesenchymal transition (EMT) has been shown to evade ADT in PCa, causing a loss of epithelial and gain of mesenchymal traits, which enhance invasiveness. The aim of this study was to investigate the relationship between AR signaling and EMT in SDC as a possible therapy evasion mechanism.

Methods
In vitro experiments were carried out on previously validated isogenic SDC cell lines and AR-/HER2-transduced NIH-3T3 cells, while in vivo experiments were performed on previously validated SDC patient derived-xenografts (PDX). In vitro protein expression was evaluated through western blot and immunofluorescence. Cell motility was assessed with migration assays and evaluated using QuPath. Bulk RNA sequencing was conducted on cell lines after dihydrotestosterone (DHT) and on PDX tumors after AAT.

Results
In vitro, it was shown that expression of AR promoted an epithelial phenotype by stabilizing E-Cadherin (E-Cad) expression, while suppressing Vimentin (Vim) and ZEB1. Conversely, the absence of AR signaling led to a mesenchymal phenotype, suppressing E-Cad and expressing Vim. After DHT stimulation these findings remained robust. AR+/HER2- cells also showed significantly lower migrative activity. In vivo, AAT caused an upregulation of pro-EMT and pro-metastatic pathways.

Conclusions
AR signaling maintains an epithelial phenotype and suppresses EMT in SDC. Loss of AR function through AAT promotes EMT-related transcriptional changes, suggesting EMT as a mechanism of therapy evasion.

Fig. 1) In vitro protein analysis of AH42 and trandsuced 3T3 cell lines



ZEB1 Immunofluorescence
in AH42 and transduced 3T3 cell lines

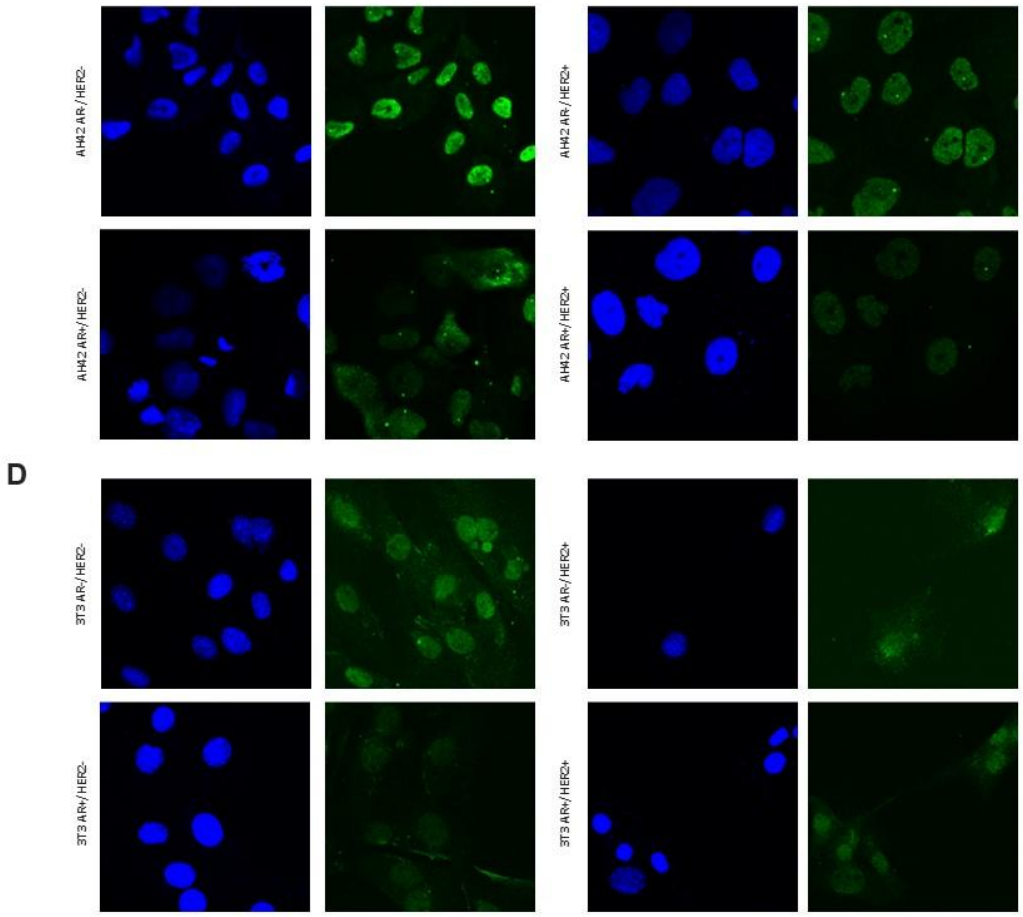
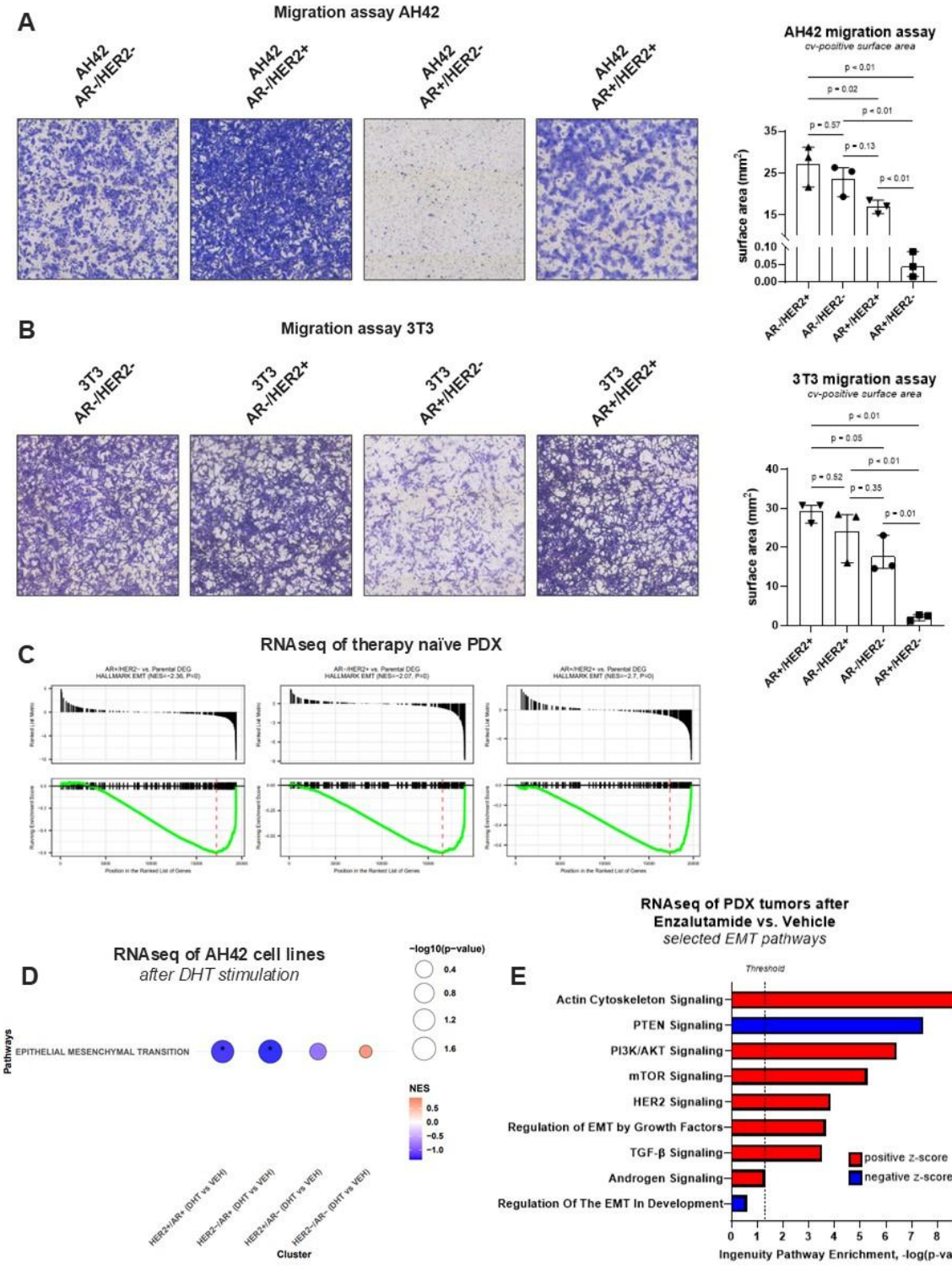


Fig. 2) Functional assessment of metastatic properties in vitro and in vivo



Nicotine-Induced SLPI Upregulation Blocks HPV Entry via Annexin A2: Mechanistic Evidence Explaining HPV-Positivity in Non-Smokers and HPV-Negativity in Smokers with Head and Neck Cancer

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Question
Head and neck squamous cell carcinoma (HNSCC) shows a paradoxical association with smoking and HPV status: HPV-positive tumors predominantly occur in non-smokers, whereas smokers mostly develop HPV-negative cancers. The underlying biological mechanism remains unresolved. This study aimed to elucidate whether nicotine-induced upregulation of the secretory leukocyte protease inhibitor (SLPI) interferes with HPV cell entry through competitive binding to Annexin A2 (AnxA2), a key host factor for viral attachment.

Methods
HaCaT and HeLa cells were transfected with specific shRNAs to downregulate SLPI or AnxA2, and exposed to nicotine at defined concentrations. SLPI and AnxA2 expression were quantified by RT-qPCR and ELISA, cytotoxicity was assessed by LDH assay, and HPV16 pseudovirion (PsV) uptake was measured using Gaussia luciferase reporter assays.

Results
Knockdown of SLPI significantly increased HPV16 PsV uptake, whereas AnxA2 knockdown strongly reduced viral entry, confirming their opposing roles in HPV infection. Nicotine exposure led to a dose-dependent increase in SLPI expression at both mRNA and protein levels without affecting cell viability, accompanied by a marked reduction in HPV16 PsV uptake. AnxA2 expression remained largely unchanged.

Conclusions
Nicotine induces SLPI overexpression, which competitively binds to AnxA2 and prevents HPV from entering epithelial cells. This mechanism provides a biological explanation for why smokers predominantly develop HPV-negative HNSCC, while non-smokers, with lower SLPI levels and more unbound AnxA2, are prone to HPV-positive tumors. The findings bridge epidemiological data and molecular mechanisms, suggesting a paradigm shift in understanding virus–host–environment interactions in head and neck carcinogenesis.

Translational Tumor Immunology and Cancer Biology

Augmenting Anti-Tumor Immunity in HNSCC by Targeting the Redox Balance of Tumor Cells

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HNSCC has a poor prognosis and immunotherapy demonstrates limited efficacy, with the metabolic microenvironment considered as an important factor mediating immune escape and therapy resistance. In our HNSCC cohort, low glutamine levels were observed in 40% of the samples. Given that glutamine is a precursor of the central antioxidant glutathione, manipulation of the fragile redox balance in tumor cells may offer a promising target to augment anti-tumor immunity and thereby the efficacy of immunotherapies.

Metabolite distribution was analyzed using mass spectrometry imaging. Effects of BPTES were investigated on PCI15 tumor cells, co-cultures of immune cells and tumor cell spheroids and fresh ex vivo tumor fragments. Spheroid lysis was monitored using the Incucyte. ROS levels and cytokine production were measured by flow cytometry.

Glutamine levels correlated positively with glutamate levels, which in turn co-localized with glutathione. Glutamine restriction increased intracellular ROS levels, limited proliferation but had no impact on viability of HNSCC cells. The administration of BPTES, inhibiting the conversion of glutamine into glutamate, the direct precursor of glutathione, exhibited effects analogous to those of glutamine restriction. Cell proliferation was restored by the addition of glutamate or glutathione, underlining the crucial importance of glutathione-mediated redox balance. Importantly, BPTES administration increased immune cell-dependent lysis of tumor spheroids and significantly enhanced response to anti-PD-1 blockade in *ex vivo* tumor fragments.

Taken together, these findings underscore the pivotal role of glutamine for ROS defense in HNSCC cells. Blocking the conversion of glutamine to glutamate perturbs the redox balance of tumor cells, rendering them vulnerable to T cell mediated anti-tumor immunity and lysis. Thus, targeting the tumor redox balance might be a promising approach to augment patient response to immunotherapy.

Nanobody-based CAR-T Cells Against HNSCC: Targeting Nectin-4 and Tackling Tumor Escape with Tandem Strategies

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Question
Head and neck squamous cell carcinoma (HNSCC) remains a major global health burden with poor prognosis despite multimodal therapies. CAR T-cell therapy has shown remarkable efficacy in treatment of hematologic malignancies of the B-cell lineage but only limited success in solid tumors, mainly due to antigen heterogeneity and an immunosuppressive tumor microenvironment. Here, we investigated whether Nectin-4 is a suitable antigen in HNSCC and whether nanobody-based mono- and tandem CAR T-cells can overcome structural and functional limitations of conventional scFv-based CARs. For tandem targeting, the immune checkpoint molecule B7-H3 was included as an additional antigen due to its high expression on both, solid cancer cells and cancer-associated fibroblasts (CAFs) but minimal presence in healthy tissue.

Methods
Nectin-4 expression was analyzed in 19 HNSCC cell lines, healthy and dysplastic oral keratinocytes, and CAFs by flow cytometry. Nectin-4 CAR constructs containing scFv or nanobody domains were expressed on human T-cells via lentiviral transduction. Functional assays compared cytotoxicity and specificity of scFv- and nanobody-based mono- and bispecific (Nectin-4/B7-H3) CAR T-cells.

Results
Nectin-4 was highly expressed in HNSCC but absent in healthy controls. Both scFv- and nanobody-based Nectin-4 CAR T-cells efficiently lysed solid tumor cells in an antigen-dependent manner. Nanobody-based CAR T-cells showed higher transduction efficiency, stable surface expression, and potent cytotoxicity. Tandem nanobody CAR T-cells effectively targeted Nectin-4 and B7-H3 simultaneously without the functional loss or structural instability observed for scFv-based tandem CAR constructs.

Conclusions
Nectin-4 is a promising novel target for HNSCC. Nanobody-based CAR T-cells offer a compact and effective next-generation platform with enhanced stability and dual-targeting capability against Nectin-4 and B7-H3, addressing key barriers in solid tumor immunotherapy.

Translational Tumor Immunology and Cancer Biology

G-CSF/NAMPT–Mediated Neutrophil Dysfunction Drives Gram-negative Infection Risk and Poor Outcomes in Head and Neck Cancer

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Bacterial infections are a leading cause of delayed therapy and early non-cancer mortality in cancer patients, despite advances in oncologic management. In a prospective cohort of head and neck carcinoma (HNC) patients, carriage of Gram-negative pathogens associated with elevated tumor-derived granulocyte colony-stimulating factor (G-CSF) in the oral cavity strongly predicted two-year infection risk and worse clinical prognosis, irrespective of traditional risk factors.

Neutrophil function, phenotype, and antibacterial responses were evaluated in saliva samples from HNC patients and matched controls using flow cytometry and microbiological assays. Mechanistic insights were explored with G-CSF–engineered murine tumor models and pharmacologic targeting of the G-CSF/NAMPT/NAD+ axis, assessing neutrophil proteome, maturation, activity, and infectious burden.

Mechanistically, chronic tumor-derived G-CSF in a NAMPT/NAD+ dependent manner reprogrammed neutrophil granulopoiesis at progenitor and mature stages, leading to cytoskeletal defects, reduced phagocytosis, and defective NET formation, while increasing tissue toxicity and impeding bacterial clearance. Importantly, long-term alterations in neutrophil progenitors induced by tumor-derived G-CSF persisted even after G-CSF withdrawal, demonstrating durable reprogramming with implications for infection risk beyond acute oncologic interventions. Pharmacological blockade of the G-CSF/NAMPT signaling axis restored neutrophil function and improved infection outcomes in vivo, highlighting a clinically actionable therapeutic strategy.

These findings identify persistent, tumor-induced neutrophil dysfunction as a central mechanism underlying poor infection control and adverse outcomes in cancer hosts. Early identification and mitigation of neutrophil impairments, particularly by targeting the G-CSF/NAMPT pathway, represent promising approaches to reduce morbidity, prevent therapy delays, and improve survival in cancer care.

Matrix-Free Patient-Derived Organoids as a Translational Platform for Personalized Immunotherapy Testing in Head and Neck Cancer

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Question
Predicting individual responses to immune checkpoint inhibitors (ICIs) in head and neck cancer remains challenging. We aimed to develop and validate patient-derived organoids (PDOs) as a functional ex vivo platform to model tumor–immune interactions and predict ICI responses.

Methods
Matrix-free PDOs were established from 38 HNSCC samples (35 patients; 63% fresh, 37% biobanked) to preserve native tumor architecture. PDOs were analyzed histologically and immunophenotypically, then co-cultured with peripheral blood mononuclear cells (PBMCs) under T cell–supportive conditions. Anti–PD-1, anti–LAG-3, and combination checkpoint blockade were applied to evaluate functional immune activation and tumor killing.

Results
PDOs formed in 75% of fresh and 71% of biobanked samples within two weeks and remained viable for up to six weeks. Histopathology and immunofluorescence confirmed structural and phenotypic similarity to matched primary tumors, retaining EpCAM, pan-cytokeratin, and Ki-67 expression. T cell–mediated tumor killing was verified in 44% of cases and was accompanied by secretion of Th1/Th17 cytokines including TNF-α, FasL, perforin, IFN-γ, and IL-17A. In parallel, elevated IL-1β and IL-10 suggested counter-regulatory mechanisms. ICI treatment reduced regulatory T cells and exhaustion markers such as PD-1 and TOX, while enhancing $CD4^+IFN\gamma^+$ and $CD8^+TOX^+$ cytolytic subsets. Responses were patient-specific and mirrored clinical outcomes.

Conclusions
Matrix-free PDOs can be generated from fresh and cryopreserved HNSCC tissue while retaining tumor architecture and molecular markers. Their co-culture with autologous immune cells provides a functional, patient-specific platform for modeling ICI responses in HNSCC.

Translational Tumor Immunology and Cancer Biology

Comparing and Combining Xevinapant with ATR and PARP Inhibition for the Radiosensitization of HPV-Negative HNSCC Cells

J. Roehrle[1], F. Gatzemeier[2,1], A. Perugachi-Heinsohn[2,1], S. Christiansen[2,1], C. Petersen[2], K. Rothkamm[2], C. Betz[1], M. Kriegs[2], H. Zech[1], T. Rieckmann[2,1]

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Question
Radiochemotherapy with the apoptosis stimulator xevinapant demonstrated superiority over radiochemotherapy in a randomized phase 2 trial in head and neck squamous cell carcinoma (HNSCC) but the following phase 3 trial failed. Here, we compared the radiosensitization through xevinapant to two other emerging approaches, the inhibition of ATR and PARP through tuvusertib and olaparib, in a panel of four radioresistant HPV-negative HNSCC cell lines (HSC4, UT-SCC-60a, SAS, SAT).

Results
Without irradiation the analyses of proliferation and colony formation showed varying sensitivities of the cell lines towards the different agents but rarely suggested more than additive effects. When combined with radiation in colony formation assays, we partly observed moderate radiosensitization through xevinapant but clearly stronger effects through tuvusertib and olaparib. Combining the latter resulted in especially profound radiosensitization in 3 out of the four cell lines, whereas their combination with xevinapant or combining xevinapant with cisplatin was less effective. Assessment of cell death induction via annexin V/DAPI staining failed to generally predict cytotoxicity or radiosensitization of these approaches.

Conclusion
Overall, our data are in line with the recent failure of the phase 3 TrilynX trial and suggest further investigation of ATR and PARP inhibition for the curative treatment of HPV-negative HNSCC.

Fig. **1**

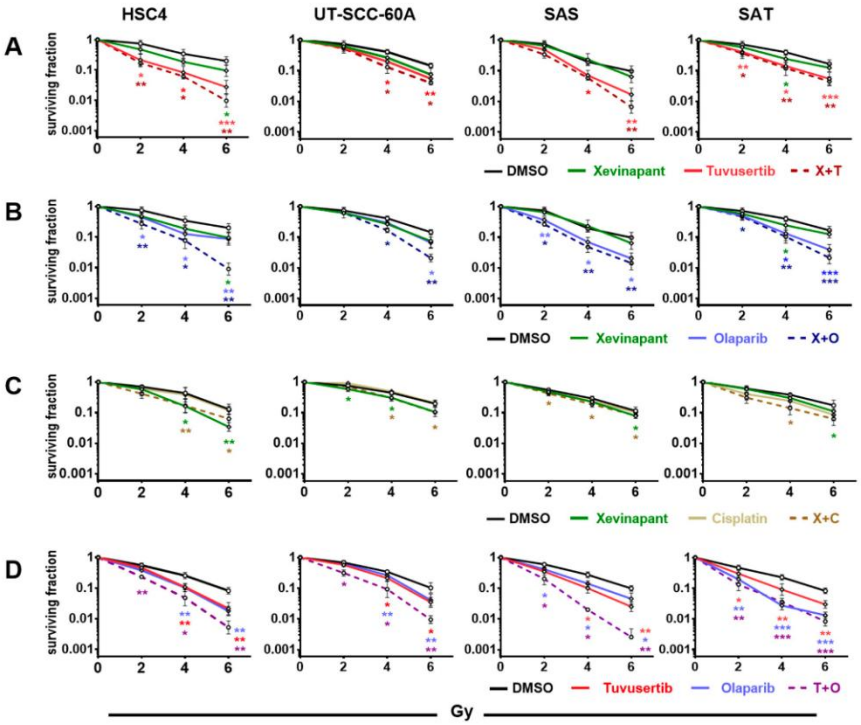

HSC4
UT-SCC-60A
SAS
SAT
A
B
C
D
surviving fraction
DMSO
Xevinapant
Tuvusertib
X+T
Olaparib
X+O
Cisplatin
X+C
T+O
Gy

Fig. **2**

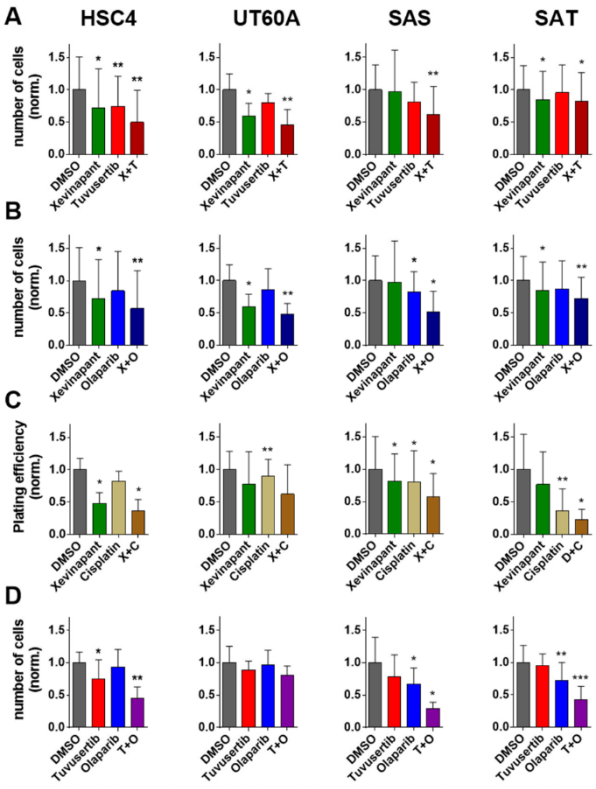
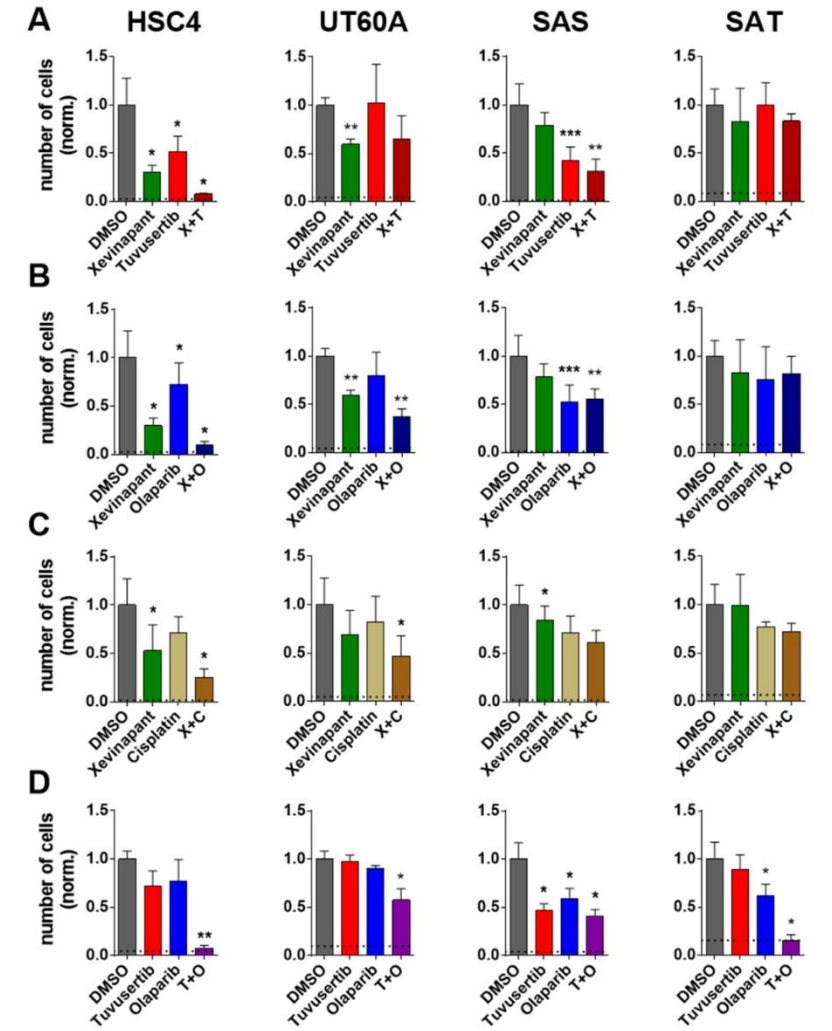

A
HSC4
UT60A
SAS
SAT
B
C
D
number of cells (norm.)
Plating efficiency (norm.)
DMSO
Xevinapant
Tuvusertib
X+T
Olaparib
X+O
Cisplatin
X+C
D+C
T+O

Fig. 3


A
HSC4
UT60A
SAS
SAT
number of cells (norm.)
DMSO
Xevinapant
Tuvusertib
X+T
B
Olaparib
X+O
C
Cisplatin
X+C
D
T+O

Translational Tumor Immunology and Cancer Biology

Dynamics of Circulating Immune Cells during Neoadjuvant Immunotherapy of HNSCC

H. Winkel[1], K. Bruderek[1], M. Pogorzelski[2], B. Höing[1], E. Deuß[1], C. H. Kürten[1], C. Doreth[1], I. Grünewald[3], M. Schuler[2,4], S. Lang[2], S. Kasper-Virchow[2,4], S. Brandau[1,4]

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Neoadjuvant Immunotherapy for head and neck squamous cell carcinoma (HNSCC) has gained increasing interest in recent years and showed promising results in clinical trials. However, the dynamics of therapy induced changes in immune response and the immunological determinants of clinical response are still underexplored.

We performed neoadjuvant treatment using PD-L1 blockade in operable HNSCC (NCT04939480). The patients received one dose of antibody three weeks before tumor resection. For our translational analyses, we collected blood samples before the therapy, weekly between antibody treatment and tumor resection, and 4 weeks after tumor resection. Pre- and post-treatment tumor samples were obtained.

5 out of 20 patients showed a major pathological response, 13 patients showed a partial pathological response and 2 patients did not respond to the therapy. In addition, 7 out of 15 patients with pre-treatment lymph node metastasis showed a reduced number of metastases, including 5 patients that had a complete regression of all metastases.

Our immunophenotyping of circulating immune cells revealed an increase in monocyte and T cell activation starting at day 15 after antibody treatment. This was accompanied by a peak of serum IFNγ and type-II IFN inducible chemokines. A differential analysis between therapy responders and non-responders revealed a higher frequency of circulating exhausted and activated CD8+ T cells, along with gamma-delta T cells, in responders. This difference was measurable before therapy and during the course of the therapy, suggesting the need for a broad pre-existing anti-tumor immune response for therapy success, which is then further augmented by the therapy.

Our study shows a detailed temporal delineation of immunotherapy induced changes in the peripheral blood immune profile of patients with HNSCC. This can guide the better timing of therapy decisions in the neoadjuvant treatment setting for this tumor type.

Precision Diagnostics and Biomarkers

Benchmarking and Optimizing Two- and Three-Dimensional Cell- and Tissue Culture Techniques to Retain the Original Tumor Heterogeneity of Primary Head and Neck Squamous Carcinomas (HNSCC) for in vitro Chemosensitivity Assays and Personalized Medicine

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Question
The in vitro culture of patient-derived primary cell suspensions presents significant technical challenges in HNSCC. Tissue dissociation protocols and two- and three-dimensional (2D or 3D) cell culture methods have a profound impact on the heterogeneity of cells retained in cultures, and affect how quickly and accurately established ex vivo tumor cell cultures become available for in vitro chemosensitivity assays and personalized medicine.

Methods
We have employed scaffold-based and scaffold-free cell and tissue culture techniques in 2D and 3D conditions, utilizing various media compositions and combinations of growth factors, to culture original cell suspensions isolated from fresh tumor biopsies via different enzymatic isolation methods. The heterogeneity of 2D and 3D cultures was analyzed by a combination of droplet microfluidics followed by single-cell RNA sequencing (scRNA-seq) and bioinformatic analyses.

Results
Both the composition of hydrogels and the combination of media, supplements, glucose, serum concentration, and growth factor combinations yielded fundamentally different cellular compositions in the resulting in vitro cultures. Furthermore, cell seeding techniques—either embedded within the gels or seeded on top of hydrogels —ideally provide the cultures with an air-liquid interface (ALI), resulting in striking changes in cells that are retained after 7-10 days of culture.

Conclusions
Our analyses revealed that the composition of the 3D scaffold, combined with media composition, growth factor combinations, and topical versus embedded cell seeding, resulted in significant differences in the heterogeneity of the in vitro cultures compared to the primary tissue. We also show that dynamic cell populations, such as cancer stem cells, subpopulations of cancer-associated fibroblasts (CAFs), and cells that undergo a partial epithelial-to-mesenchymal transition (pEMT) can be retained, using suitable cell culture conditions, but also squamous cancer cells. Providing a functional ALI also affects the retention of immune and endothelial cells in culture.

Cell-free Circulating Tumour DNA Analysis in Patients with HNSCC: Liquid Biopsy for Therapy Planning in Clinically N0 Head and Neck Cancer (LiC0HN) Study

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In patients with clinically negative cervical lymph nodes (cN0), therapeutic strategies remain a subject of ongoing debate. ctDNA may improve detection of occult metastases and reduce indications for surgery. In our previous LIONESS study, we demonstrated the potential of whole exsome sequencing-informed personalized ctDNA analysis as a marker for molecular residual disease following curative-intent surgery, highlighting potential role in guiding treatment decisions for HNSCC patients.

We conducted a prospective cohort study (LiC0HN) to evaluate optimal strategies for ctDNA detection in cN0 HNSCC. Seventy-five patients with stage I–IV HNSCC undergoing primary curative surgery provided samples, including tumour tissue, plasma, buffy coat, and dried blood spots (DBS). Objectives were to: (1) evaluate whether whole genome sequencing (WGS)-informed ctDNA detection reflects nodal metastasis and postoperative tumour clearance, (2) detect ctDNA in DBS via copy number alterations, and (3) identify tumour/nodal epigenetic markers for methylation-based ctDNA assays. WGS was performed on cfDNA from plasma and DBS, with matched tumour and germline DNA. Additionally, whole methylome sequencing was conducted on FFPE tumour and lymph node tissue using enzymatic library preparation.

Copy number alterations were detected in both pre- and post-operative plasma samples from a subset of patients using both tumour-agnostic and tumour-informed approaches. Similarly, tumour-informed WGS analysis enabled ctDNA detection in a subset of DBS samples. Methylation analysis identified 24 shared differentially methylated regions between primary tumours and matched nodal metastases, with 22 CpG blocks showing consistent directional changes. Many of these regions are associated with genes involved in cell adhesion and metastasis.

The findings of our study highlight the potential of ctDNA analysis to inform clinical decision-making and improve outcomes in surgically treated patients with HNSCC.

Precision Diagnostics and Biomarkers

A cfDNA-methylation-based Prognostic Indicator for Pathological Response after Neoadjuvant Immunotherapy for HNSCC

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Introduction
Immune checkpoint inhibition (ICI) has revolutionized oncology. Unfortunately, patients with head and neck squamous cell carcinoma (HNSCC) are still awaiting the perspective-changing benefits of this therapeutic modality, despite promising data in neoadjuvant settings. Radiological assessment of tumor response to ICI is challenging as it fails to account for tumor viability – complicating its clinical application. The clinical utility of cfDNA-methylation marks as an indicator for pathological response following ICI were evaluated.

Methods
Plasma samples from 20 patients, who received PDL1-checkpoint-Inhibitor Atezolizumab three weeks before curative surgery as part of a single-arm, Phase-II-study (PIONEER, EudraCT-Number: 2018-000254-21), were analyzed. cfDNA-methylation profiles derived from samples acquired at different timepoints were evaluated in relation to immune-related pathological response data to identify response cfDNA-predictors.

Results
Many DMPs (differentially methylated positions) showed significant differences between responders and nonresponders to neoadjuvant ICI. The promotor region of a variable part of the beta chain of the T-cell-receptor (TRBV6-1) was significantly hypomethylated pretherapeutically in the responder group ($p = 0{,}0019$). Posttherapeutically, however, there was no significant difference between responders and nonresponders regarding methylation of the promotor region of TRBV6-1.

Conclusion
This study aims to establish cfDNA-methylation as a non-invasive tool for the stratification of HNSCC patients in view of ICI treatment and monitoring disease progression to inform timely interventions and guiding personalized therapy approaches. Further studies focussing on both cfDNA-methylation and DNA-methylation from tumor tissue should be conducted.

Acknowledgements This research was made possible through the imCORE Network supported by F. Hoffmann-La Roche.

Precision Diagnostics and Biomarkers

HPV Integration as a Prognostic Marker in Oropharyngeal Cancer: Proximity-Ligation Sequencing (TLC) Enables FFPE-Based Detection and Molecular Risk Stratification

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Background
Human papillomavirus (HPV)-associated oropharyngeal squamous cell carcinoma (OPSCC) represents a biologically distinct subgroup with generally favourable prognosis. Nevertheless, up to 25 % of HPV-positive patients develop relapse or metastasis, indicating biological heterogeneity. Integration of the viral genome into the host DNA is considered a key step in tumour progression, but comprehensive analysis has long been hindered by the poor suitability of formalin-fixed, paraffin-embedded (FFPE) tissue for sequencing approaches originally designed for fresh-frozen material.

Methods
We established and validated a proximity-ligation-based sequencing workflow, Targeted Locus Capture (TLC), allowing robust HPV integration detection directly from routine FFPE samples. The method combines formalin-induced crosslinking with targeted enrichment and next-generation sequencing to identify viral-host junctions, copy number changes, and structural variants across hundreds of kilobases.

Results
In 27 HPV-positive OPSCCs, HPV integration was identified in 56 % of cases. Integration events were frequently associated with complex genomic rearrangements, including deletions, duplications, and inversions at cancer-relevant loci such as TP63. The results demonstrate high concordance with previous reference data and highlight the analytical reliability of TLC for degraded FFPE DNA.

Conclusion
Proximity-ligation sequencing enables precise HPV integration mapping in routinely processed tumour specimens, overcoming the limitations of fresh-frozen-dependent methods. The technology is now ready for large-scale application in an ongoing DFG-funded study to evaluate the prognostic and biological significance of HPV integration in OPSCC.

Precision Diagnostics and Biomarkers

Tumor – Educated Platelets as a Liquid Biopsy Tool in Head and Neck Cancer

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Objective
Research has shown that changes in the RNA profile of tumor – educated platelets (TEPs) may indicate the presence of tumors. In a recent study, a TEP RNA signature was identified utilizing two independent cohorts of patients with head and neck squamous cell carcinoma (HNSCC). Therefore, the aim of this study was to evaluate the diagnostic potential of TEP RNA, with a particular focus on RNAs encoding *COL1A1* (collagen type I, alpha I), *ZNF750* (zinc finger protein 750), *Ly6D* (lymphocyte antigen 6 family member D), *WNT5a* (WNT family member 5a) and *KRT5* (keratin 5) for the diagnosis and tumor staging of HNSCC. An analysis of specific TEP RNA occurrence with tumor localization and HPV status (human papillomavirus) was also performed.

Methods
Platelets were isolated from tumor patients (TPs, n = 40, representing T1 – T4 with each n = 8 and HPV+ = 8) and healthy donors (HDs, n = 10), followed by RNA extraction and quantitative reverse transcription – polymerase chain reaction (qRT – PCR) analysis.

Results
Increased levels of *COL1A1*, *WNT5a*, and *KRT5* RNA were detected in samples from patients with T2 and T3 tumors compared to those from healthy subjects. TEP – derived RNAs encoding *COL1A1*, *ZNF750*, *KRT5* and *WNT5a* were also more prevalent in platelets of patients diagnosed with lymph node stages N1 and N2. In addition, *COL1A1* and *WNT5a* RNA were more abundant in platelets from patients suffering from oral and oropharyngeal carcinoma.

Conclusion
These results confirm the assumption that the platelet RNA profile differs between HNSCC patients and healthy individuals, but further research is needed to implement TEP RNA analysis as a reliable liquid biopsy tool in routine cancer diagnostics.

Circulating Pathologically Activated Neutrophils are Reduced after Curative Therapy and may serve as a Biomarker of Response during Neoadjuvant Immunotherapy

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Background
Neutrophils are increasingly recognized for their immunomodulatory roles in cancer. We previously demonstrated that pathologically activated neutrophils—low-density neutrophils (LDNs)—can be detected in the circulation of cancer patients and comprise functionally distinct immature and mature subsets. Importantly, the mature subset exerts potent T-cell suppressive activity, implicating these cells in shaping antitumor immunity. Building on this finding, we investigated how LDN subset dynamics evolve under curative therapy and neoadjuvant immunotherapy.

Methods
We analysed 55 patients receiving standard of care treatment (surgery / surgery + radio-chemotherapy) and 20 patients enrolled in a neoadjuvant PD-L1 checkpoint blockade trial (NCT04939480). Density gradient separation and flow cytometry-based immunophenotyping were performed to distinguish immature from mature LDN subsets. Samples were collected longitudinally to assess baseline levels and therapy-induced changes. Clinical response was defined as a pathological tumor-reduction of more than 50%.

Results
In the standard-of-care cohort (n = 55) pre-treatment levels of LDNs declined after therapy, with the most pronounced reduction observed for the mature suppressive LDN subset. In the neoadjuvant immunotherapy cohort (n = 20) non-responders displayed higher pre-treatment frequencies of the mature LDN subset and an elevated mature-to-immature ratio was maintained during treatment. In contrast, responders exhibited a decline in the relative frequency of suppressive mature LDN over time.

Conclusions
Our results indicate that successful surgery-based standard-of-care treatment leads to a reduction of immunosuppressive pathologically activated neutrophils. Interestingly, this effect was also observed during the pre-operative immunotherapy window, especially in pathological responders. Thus, LDN harbor potential as early biomarker for therapy response and may inform future neoadjuvant treatment regimens.

Larynx Organ Preservation for Hypopharyngeal Cancer Patients: Non-Inferior 10-Year Ssurvival Compared to Laryngeal Cancer after Induction Chemotherapy and Cetuximab

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Introduction
Locally advanced hypopharyngeal squamous cell carcinoma (LA-HSCC) has unfavorable prognosis for several reasons. Multimodal treatment approaches include ablative surgery up to total laryngectomy (TL), followed by postoperative radio(chemo)therapy (POR(C)T), induction chemotherapy (IC) followed by radiotherapy (RT) or primary radio(chemo)therapy. The exclusion of hypopharyngeal cancer patients from larynx organ preservation (LOP) trials due to deviating biology and presumed inferior survival compared to laryngeal cancer is widely discussed. The LOP trial DeLOS-II included both subsites and long-term survival data is now available for comparing larynx and hypopharynx subsites.

Methods
DeLOS-II used TPF-IC plus RT ± cetuximab. Responders with endoscopically estimated tumor surface shrinkage ≥ 30% after cycle 1 (IC-1) received further 2 cycles IC+RT. Non-responders underwent TL+POR(C)T. Follow-up data from 52 DeLOS-II patients (30.1% among 173 patients of the DeLOS-II patients treated in our hospital) were analyzed using SPSS.

Results
Of these 52 patients, 30 had tumors located in the hypopharynx, and n=15 HSCC patients received cetuximab, of whom n=7 (46.7%) were still alive after 125 months. After 125 months, only 3 (20%) of 15 HSCC patients who did not receive cetuximab were alive. Of the larynx cancer patients (n=22), 3 (30%) of 10 patients receiving cetuximab were alive after 125 months, whereas 3 (25%) of 12 patients without cetuximab survived. According to log-rank tests, OS and TSS did not differ between larynx or hypopharynx cancer patients (all $p > .05$).

Conclusions
Patients with LA-HSCC showed non-inferior long-term survival when treated according to the DeLOS-II protocol aiming on LOP. The possibility of a positive effect of cetuximab cannot be ruled out.

Evaluation of Confocal Laser Endomicroscopy as a Tool for Optical Biopsy in Nasal Cavity Carcinomas

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Question
In the surgical management of nasal cavity carcinoma (NCC), oncological safety requires *in sano* resection, yet the area is functionally and aesthetically sensitive. Even a few millimeters of tissue can critically affect postoperative quality of life. An optical biopsy enabling *in vivo* assessment at resection margins is therefore desirable. Confocal Laser Endomicroscopy (CLE), originally developed for endoscopy in gastroenterology, has been tested by our group in other head and neck squamous cell carcinomas.

Methods
68 CLE video sequences (4,500 images) with histological correlation (tumor or healthy tissue) were randomized and blindly reviewed by three experienced examiners. Each provided a malignancy assessment and completed a previously validated scoring system. NCC-specific cut-offs were derived, and examiner-based assessments were systematically compared with score-based classifications. Interrater agreement between expert assessments and score-based evaluations was analyzed.

Results
Examiner-based assessment showed sensitivity 86.3% ± 9.1 and specificity 94.1% ± 6.4, with PPV 93.4% ± 7.3 and NPV 87.5% ± 8. Score-based analysis achieved sensitivity 94.1% ± 4.8 and specificity 82.4% ± 18.8. Interrater agreement between expert assessments and score-based evaluations was substantial (Fleiss" $\kappa = 0.75$).

Conclusion
These first results are promising, suggesting that CLE can reliably assess NCC *in vivo*. Larger studies directly comparing CLE with frozen section analysis are needed before routine clinical implementation. The scoring system was reliable and could support less experienced examiners, although this potential requires further validation.

3D Specimen Mapping to Optimize Postoperative Radiation Planning for Head and Neck Cancer

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Background
Optimal postoperative radiation therapy (PORT) for head and neck cancer (HNC) depends on accurate correlation of surgical and pathological findings, but current communication methods lack detailed spatial information. This study assessed the feasibility of using virtual 3D surgical specimens (v3DSS), annotated for margin status, to enhance PORT planning.

Methods
Ten advanced HNC specimens were 3D scanned and annotated for positive/close margins. The v3DSS were registered to preoperative and post-surgical CT scans, and volume overlap (VO) with clinical target volumes (CTV) and planning target volumes (PTV) was measured. Eight radiation oncologists (ROs) completed surveys on the utility of v3DSS in planning workflows.

Results
Mean VO between v3DSS and CTV was 70.6 ± 21.8%, and 86.9 ± 14.7% with PTV. Notably, 30% of positive/close margins were excluded from CTV. ROs rated v3DSS as more informative and clinically useful than conventional pathology reports, slides, or verbal updates across all categories ($p<0.01$).

Conclusions
v3DSS integration into PORT planning may improve margin visualization and identification of recurrence-prone areas, supporting more accurate radiation targeting. Both quantitative overlap analysis and RO feedback highlight its clinical value, warranting larger studies to evaluate its impact on treatment planning and outcomes.

A High-Resolution Micro-PET for Intraoperative Assessment of Resection Status in Malignant Tumors in the Head and Neck Region

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Introduction
Histological examination is the gold standard for diagnosis and treatment decisions in head and neck cancer patients. However, it involves a time delay and it is not uncommon for subsequent resections to be necessary to achieve a reliable R0 status. Intraoperative micro-PET could offer the possibility of checking intraoperative resection margins, complementing the diagnosis in a meaningful way, and minimizing the risk of repeat surgery.

Methods
Intraoperative micro-PET was tested for feasibility in 9 patients with head and neck tumors. FDG PET was performed in 8 patients and FAPI-PET in one patient. Tumor resection was performed with curative intent as en bloc resection with ipsilateral or bilateral neck dissection. Conventional PET was performed prior to surgery in each case. Resection specimens were placed in the micro-PET and measured. This allowed the size of the tumor and the resection margins to be determined and compared with histology and preoperative imaging.

Results
The examination was performed without complications in all patients. Compared to conventional PET and histology, comparable results were obtained with regard to tumor extent, resection margins, and sensitivity of positive cervical lymph nodes.

Conclusion
Intraoperative micro-PET provides immediate information about resection margins and the positivity of local lymph node metastases. This makes it possible to avoid additional operations and improve the long-term prognosis for patients. Studies with larger numbers of cases are to be planned in order to optimally highlight the advantages of this diagnostic tool.

Rapid Evaporative Ionization Mass Spectrometry Guided Intraoperative Tissue Classification in Head and Neck Squamous Cell Carcinoma

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Introduction
Surgical success in head and neck squamous cell carcinoma (HNSCC) depends on complete tumor resection (R0) and a major challenge remains the lack of real-time tissue assessment. Frozen section histopathology, the current standard, is limited by restricted sampling and extended operative time, which increases the risk of R1 resection. Analysis of metabolites in the surgical aerosol by Rapid Evaporative Ionisation Mass Spectrometry (REIMS) enables real-time intraoperative tissue classification and may overcome these limitations.

Methods
We aimed to optimize REIMS-guided surgery. During nine HNSCC surgeries, aerosols generated by monopolar electrosurgery or CO2 laser were aspirated into a spectrometer with a REIMS ion source and data from healthy and tumor tissue were generated in vivo and ex vivo. Sampling conditions, tube configuration, suction strength, and dilution gas flow were optimized to maximize signal-to-noise ratio. Spectra were annotated under surgical supervision and used to construct tissue classification.

Results
The REIMS setup was successfully integrated without interfering with the surgical workflow. Metabolic differences between tumor and non-tumor tissues were evident, with linear discriminant analysis model building in the range of 50-1000 m/z incorporating small metabolites, fatty acids and phospholipids showing distinct separation between tumor and non-tumor tissue. Single spectra of tissue revealed specific fingerprints of tissue types in the 600-900 m/z phospholipid region through different peak distributions and ratios.

Conclusion
This pilot study confirms the feasibility of REIMS-guided surgery in HNSCC. It enables intraoperative differentiation between tumorous and non-tumorous tissue and provides the basis for future large-scale data modelling, allowing intraoperative tissue annotation. Therefore, the approach offers real-time margin assessment with the potential to improve surgical precision, R0 rates and reduce operative time.

Innovative Technologies for Precision Treatment

When Tumor Meets Host: Tracking Therapy Responses in Head and Neck Cancer Slice Cultures

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Objective
The aim of this study was to establish an ex vivo model of head and neck squamous cell carcinoma (HNSCC) that preserves tumor–host interactions and enables the assessment and prediction of therapy responses.

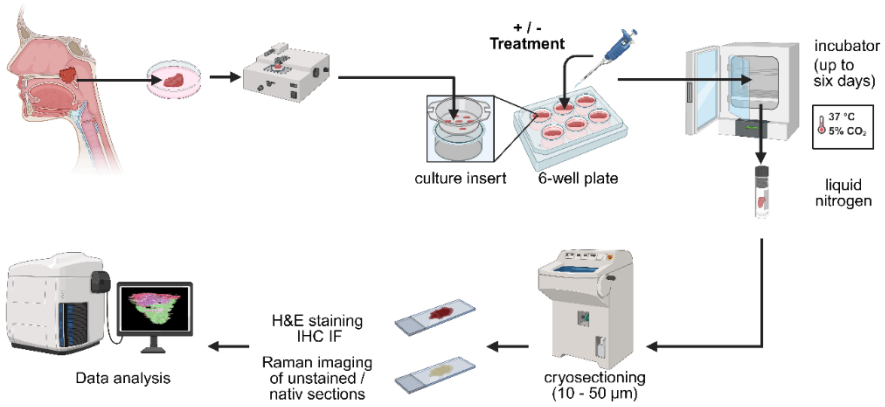
Methods
Patient-derived HNSCC biopsies were cut into 350 µm slices and cultured up to six days. Slices were treated with cisplatin or atezolizumab (PD-L1 antibody) and responses were assessed via immunohistochemistry (IHC, immune infiltration, apoptosis, proliferation) and Raman spectroscopy to correlate biochemical and histopathological changes.

Results
Thick-slice cultures preserved native tumor architecture and microenvironment. Cisplatin and PD-L1 antibody treatment induced significant apoptotic and immune responses compared to untreated controls. Raman spectroscopy detected distinct, therapy-related biochemical shifts correlating with IHC markers of treatment response and immune activation, confirming the model"s pharmacological sensitivity

Conclusion
The present findings demonstrate that ex vivo thick-slice cultures preserve essential tumor–host features and respond sensitively to therapeutic interventions. The changes can partly also be monitored by Raman spectroscopy. This may allow a real-time biophotonic monitoring of the slice cultures without the need of IHC in the future. The results are highly promising, suggesting that this approach could serve as a reliable and physiologically relevant platform to investigate tumor–host dynamics and assess personalized therapy responses in HNSCC. Further validation in larger cohorts is ongoing.

Figure 1: Overview of tumor tissue slice culture: 350 µm sections were cultured up to six days, with samples collected at various time points for H&E, IHC, IF and Raman analysis.

Fig. 1



Liquid Biopsy for Diagnosis, Response Assessment & Monitoring

HPV16 E6 serology based screening and early detection of HPV-driven oropharyngeal cancer

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Objective
An increasing proportion of oropharyngeal cancers (OPC) are attributable to human papillomavirus (HPV-OPC). Serum antibodies against HPV16 early proteins and cell-free HPV DNA (cfHPV DNA) in liquid biopsies continue to emerge as valuable pre-diagnostic biomarkers. In this update, we provide follow-up data on our prospective proof-of-concept study evaluating HPV serology for early detection of HPV-OPC.

Methods
HPV16 antibodies were measured in 4,424 sera of the Hamburg City Health Study (HCHS), a population-based cohort. The following participants were enrolled into a clinical follow-up study: 1) participants seropositive for HPV16 E6 and at least one additional early protein (high risk for HPV-OPC development); 2) participants positive for E6 alone. Participants underwent 6-monthly head and neck examinations and blood draws. Suspicious lesions were evaluated by MRI and panendoscopy with biopsy. cfHPV DNA detection in blood plasma was performed by dPCR.

Results
10/12 participants considered to be at high-risk, participated in regular follow-up examinations. After 6 years of active follow-up, 5/10 were diagnosed with stage I HPV-OPC. Additional 11 of the 24 HPV16 E6 only participants attended follow-up, in which a further stage I HPV-OPC was diagnosed. This case was seropositive for a second early antigen at the time of diagnosis. 3/6 cases presented with either no or a single lymph node metastasis. cfHPV DNA was detectable at diagnosis in 5 cases at varying levels, however not in the one patient without lymph node involvement. Three cases with available pre-diagnostic blood samples showed a steep increase in cfHPV DNA levels just prior to diagnosis.

Conclusion
HPV16 serology-based screening is suited to prospectively identify HPV-OPC cases. In a future screening scenario, longitudinal biomarkers assessment may dynamically refine the risk classification.

Poster Group 1

P01

Integrated Multi-Omics Analyses of Oral Squamous Cell Carcinoma Reveal Precision Patient Stratification and Personalized Treatment Strategies

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Oral cavity squamous cell carcinoma (OSCC), a leading subtype of head and neck cancer, is characterized by high global incidence and mortality rates. Despite surgery and radiochemotherapy, nearlyone-third of patients relapse. Targeted therapies and immunotherapies for recurrent OSCC show limited effectiveness, underscoring the need for improved treatments.

To improve current treatment strategies for recurrent OSCC, we conducted multi-omics analyses on pretreatment OSCC samples (n=137) and identified A3A and EGFR as inversely expressed markers for patient stratification and response prediction. Our data demonstrated that elevated A3A or PD-L1 expression levels correlated with improved responses to anti-PD-1 therapy in patients (n=50, IHC). In contrast, high RRAS expression (n=252, qRT-PCR) was significantly associated with OSCC recurrence. Cell-based experiments revealed that RRAS was involved in radiotherapy and cisplatin resistance through the EGFR/RRAS/AKT/ERK signaling pathway. In OSCC patient-derived xenograft (PDX) mouse models, treatments with cisplatin and cetuximab effectively reduced tumor size in EGFR-high-derived but not A3A-high-derived PDX tumors. Our study demonstrated that A3A-high tumors exhibited immune-hot features and were responsive to anti-PD-1 therapy. EGFR-high tumors showed chromosome 7p11.2 gains and DNA repair alterations. OSCC PDX models confirmed that EGFR-high tumors were sensitive to cisplatin and cetuximab. Additionally, RRAS-high tumors were associated with OSCC recurrence via AKT and ERK phosphorylation Interestingly, these tumors showed improved clinical outcomes with cetuximab therapy.

This study emphasizes the significance of A3A and EGFR expression levels in OSCC patient stratification and precision therapy, suggesting the use of anti-PD-1 or anti-EGFR treatments, respectively based on these biomarkers. Furthermore, RRAS emerges as a novel prognostic marker for local recurrence.

Fig. **1**

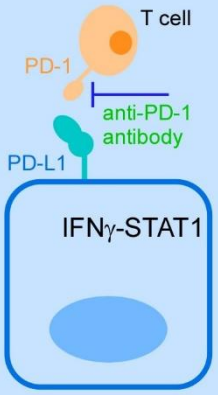
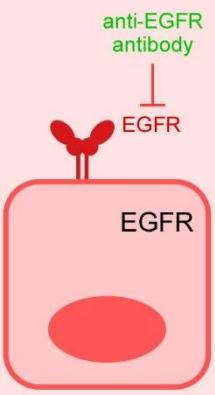
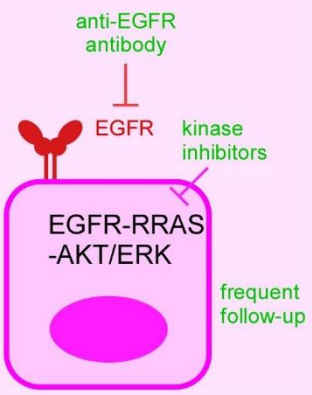
OSCC patients (Clinical samples)			
Integrated multi-omics analysis	Genomics, Transcriptomics, Proteomics, Phospho-proteomics		
Stratification	 $A3A_H EGFR_L$	 $A3A_L EGFR_H$ recurrence patients  $RRAS_H$	
Biomarkers	**APOBEC3A / PD-L1**	**EGFR**	**RRAS**
Scoring	**IHC > 150**	**IHC > 150**	**IHC > 150**
Characteristics	**High infiltration of Immune cell** (e.g., $CD8^+$ cells) **High expression of immune checkpoint genes** (e.g.,PD-L1)	**Copy number gain** Chr. 7p11.2, Chr.11q13.3, DDR genes	**High expression of recurrence genes** (e.g., RRAS, RAP1B) **Activated phosphorylation proteins** p-AKT2_S474, p-ERK1_Y204
Key pathway	**IFN-γ-STAT1**	**EGFR**	**EGFR-RRAS-AKT/ERK**
Treatment options	**Immunotherapy** anti-PD-1 antibody (e.g. Nivolumab, Pembrolizumab)	**Targeted therapy** anti-EGFR antibody (e.g.cetuximab)	**Targeted therapy** anti-EGFR antibody AKT/ERK kinase inhibitors frequent follow-up
Model			

Figure. Summary of proteogenomic analyses for patient stratification, biomarkers, significant signaling pathways, and potential treatment options for OSCC.

Poster Group 1

P02

Unique Trajectories of B Cells Within Tumor-Associated Lymphoid Structures in Head and Neck Cancer

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Background

Tumor tertiary lymphoid structures (TLS), particularly mature TLS (mTLS), are associated with enhanced antitumor immunity, yet the role of B cells within these structures remains unclear.

Materials and methods

By integrating single-cell transcriptomics and B-cell receptor sequencing in head and neck squamous cell carcinoma (HNSCC), this study reveals distinct B-cell dynamics between immature TLS (imTLS) and mTLS.

Results

Within mTLS, B cells undergo robust clonal expansion and differentiate into plasmablasts and plasma cells, accompanied by immunoglobulin class switching and extensive clonal sharing across subclasses. Conversely, B cells in imTLS predominantly remain stalled in follicle-like or FCRL4^{+} states, with limited progression toward terminal differentiation. Intercellular communication analysis further indicates that supportive interactions, including those mediated by regulatory receptors, are abundant in mature TLS but diminished in immature TLS.

Conclusion

Collectively, these findings reveal that TLS maturity is a key determinant of effective B-cell responses within the tumor microenvironment and elucidate how B-cell differentiation states shape local antitumor immune responses.

Poster Group 1

P03

In Silico Identification of EMT-Relevant Genes Associated with COL1A1 in Head and Neck Cancer

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Introduction

Epithelial–mesenchymal transition (EMT) is a key process in tumor invasion and metastasis, enabling epithelial cancer cells to acquire migratory properties. This study aimed to identify differentially expressed genes (DEGs) between head and neck squamous cell carcinoma (HNSCC) and normal samples, with a focus on EMT-associated genes and their role in tumor progression.

Methods

Gene expression data from the GSE6631 dataset (22 HNSCC, 22 normal samples) were analyzed to identify DEGs ($log2FC \geq 1.0$; $p < 0.05$). DEGs were cross-referenced with 214 EMT-related genes (GO:0001837, Gene Ontology) to detect EMT-DEGs. STRING was used for PPI network construction, and the hub genes and co-expression modules were identified through Cytoscape software using the CytoHubba and MCODE plugins, respectively. Pathway enrichment analysis related to the top co-expression module was performed with ToppGene (adjusted $p < 0.01$).

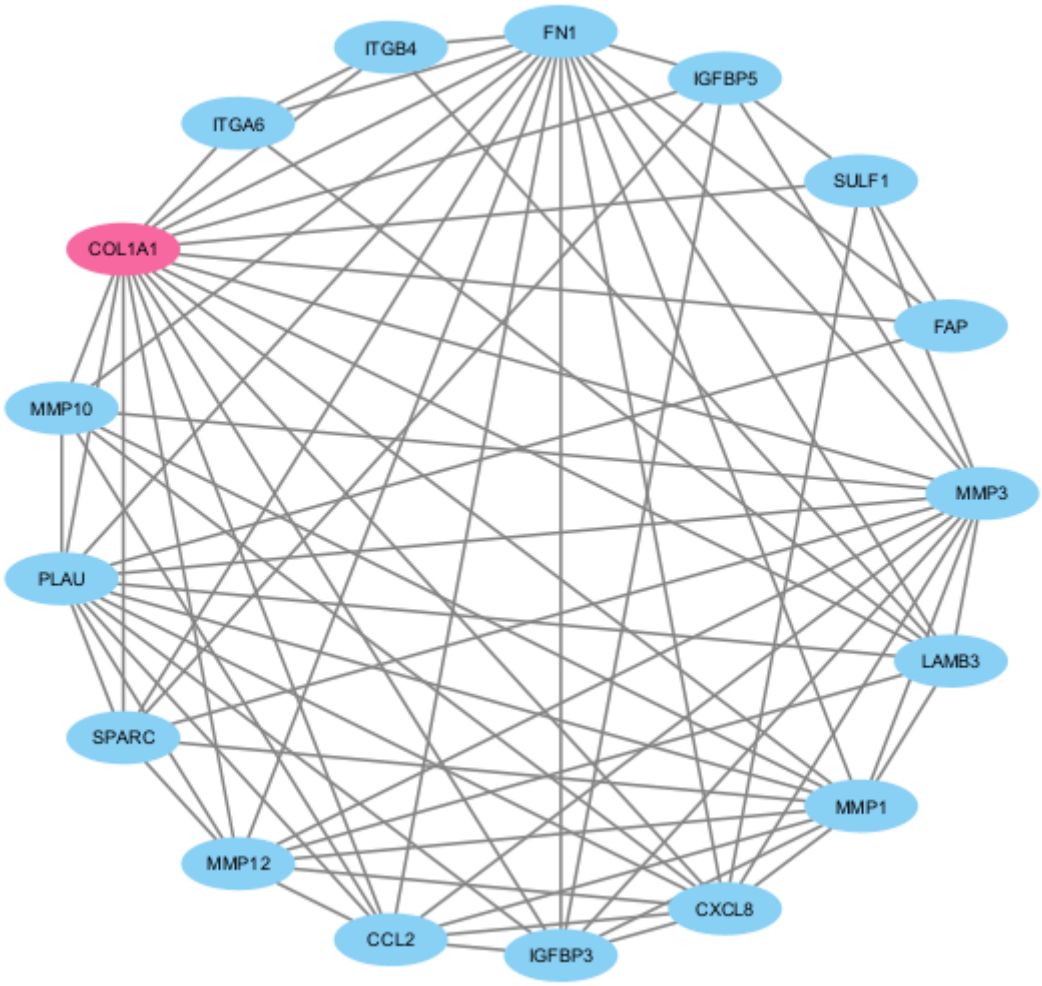
Results

Among 167 DEGs, 2 overlapped with EMT-related genes (COL1A1 and PDPN). COL1A1 ranked as the top hub gene in the PPI network constructed using DEGs.The co-expression network showed one cluster that was identified with a score of 9.25, comprising 17 nodes,including LAMB3, MMP3, FAP, ITGA6, CXCL8, FN1, CCL2, MMP10, MMP1, SULF1, PLAU, ITGB4, COL1A1, IGFBP3, IGFBP5, SPARC, and MMP12 along with 74 edges. Enrichment analysis revealed significant pathways associated with the EMT-DEGs, including ECM–receptor interaction, focal adhesion, and cancer-related pathways.

Conclusions

This study emphasized the important role of EMT-related factors in the progression of HNSCC. COL1A1 was identified as a central gene within a co-expression module enriched for EMT markers, suggesting its potential involvement in tumor invasion. These findings provide further insight into the molecular mechanisms underlying HNSCC progression and may inform future therapeutic strategies targeting EMT-associated pathways.

Fig. **1**


FN1
ITGB4
IGFBP5
ITGA6
SULF1
COL1A1
FAP
MMP10
MMP3
PLAU
LAMB3
SPARC
MMP1
MMP12
CXCL8
CCL2
IGFBP3

Poster Group 1

P04

Optimizing Cochlear Implant Candidacy in Tumor Patients: Pre- and Intraoperative Auditory Nerve Assessment with Advanced Electrophsiolocial Techniques

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Pre- and intraoperative electrically evoked auditory potentials (eAEP), including eABR, eAMLR, and eALR, are essential for assessing cochlear implant (CI) candidacy in tumor patients with uncertain auditory nerve functionality. Techniques such as PromStim, ANTS, and PromCERA provide objective evaluations of auditory pathway integrity. Preoperative tests like LA-TT-EABR and LA-TT-EALR, performed under local anesthesia, demonstrate high reliability, with sensitivity and specificity reaching 100% in some studies, avoiding unnecessary general anesthesia and enabling tailored decision-making.

Intraoperative methods, including ANTS and CI-based stimulation, confirm preoperative findings and guide surgical decisions, particularly in cases of vestibular schwannoma resection or retro-cochlear pathologies. These approaches minimize risks of unsuccessful CI outcomes. Cortical-level assessments, such as LA-TT-EALR, complement brainstem evaluations, offering insights into cortical plasticity and potential post-implantation outcomes.

Combining eAEPs with advanced imaging tools like OTOPLAN optimizes electrode placement, reducing tonotopic mismatches and enhancing CI success rates. This integrated framework ensures comprehensive evaluation and improved outcomes for tumor patients.

Fig. 1: Illustrating workflow to evaluate the auditory pathways's function.

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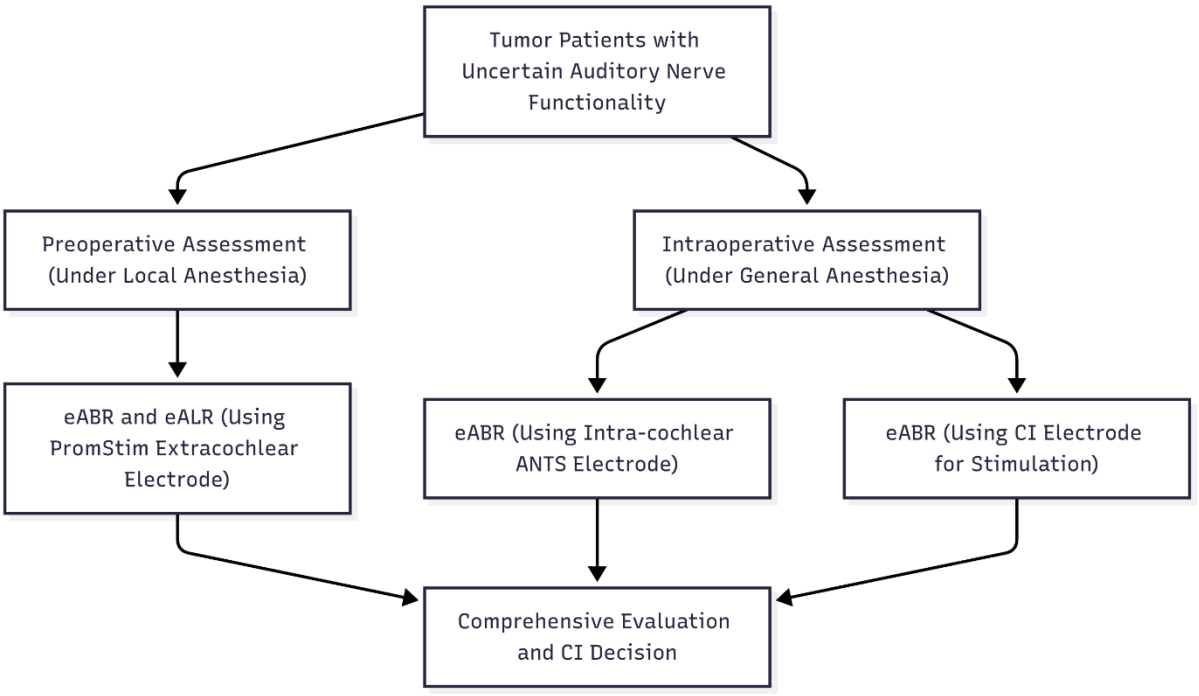
Fig. 1


Tumor Patients with Uncertain Auditory Nerve Functionality
Preoperative Assessment (Under Local Anesthesia)
Intraoperative Assessment (Under General Anesthesia)
eABR and eALR (Using PromStim Extracochlear Electrode)
eABR (Using Intra-cochlear ANTS Electrode)
eABR (Using CI Electrode for Stimulation)
Comprehensive Evaluation and CI Decision

Poster Group 1

P05

NECTIN-4 as an Actionable Vulnerability in Sinonasal Undifferentiated Carcinoma: Biomarker-Guided Enfortumab Vedotin Achieves Deep and Durable Control

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Question

Can NECTIN-4 be used as an actionable biomarker in sinonasal undifferentiated carcinoma (SNUC), and does NECTIN-4–targeted ADC therapy (enfortumab vedotin, EV) deliver durable clinical benefit when selected by multi-omic profiling?

Methods

A 66-year-old man with bilobar liver-metastatic, therapy-refractory SNUC (after platinum chemoradiation, immune checkpoint blockade, and MET inhibition) underwent NCT/DKTK MASTER profiling. Genomics showed NECTIN4 amplification with IDH2 mutation and MET amplification. IHC confirmed high NECTIN-4 protein (H-score 210/300). Off-label EV was administered 1.25 mg/kg on days 1, 8, 15 q28d, then 1.0 mg/kg. Imaging occurred every 6–8 weeks; adverse events were monitored.

Results

EV produced rapid, deep shrinkage: hepatic targets −48.8% after 3 cycles and −59.4% after 6. Treatment stopped after cycle 8 for grade 2–3 polyneuropathy. >10 weeks after discontinuation, imaging showed ongoing control (residual 29.0 mm) without new lesions. Overall benefit exceeded 11 months, including a treatment-free interval, with clinical stability.

Conclusions

Biomarker-guided EV achieved clinically meaningful and durable disease control in NECTIN-4–positive SNUC, identifying NECTIN-4 as an actionable vulnerability. Concordant gene amplification and high protein expression support target engagement and echo predictive signals from other cancers. Implications: (i) routine NECTIN-4 screening (IHC ± copy-number) across rare head-and-neck cancers; (ii) prospective, tissue-agnostic trials/registries of EV (alone or in rational combinations) in NECTIN-4–positive disease; (iii) pan-cohort multi-omic analyses across NCT/DKTK MASTER and partner H&N cohorts to derive and validate a composite NECTIN-4 benefit score (CNV, RNA, proteomics, IHC) for patient selection and resistance monitoring.

P06
Tracking Tumor Heterogeneity in Head and Neck Squamous Cell Carcinoma

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Objective
Head and neck squamous cell carcinoma (HNSCC), the sixth most common cancer worldwide, exhibits marked intra-tumoral heterogeneity and clonal diversity. HPV-positive tumors usually have a better prognosis, while HPV-negative cases—often linked to tobacco and alcohol—show more aggressive behavior. This study aims to characterize tumor heterogeneity in HNSCC across anatomical sites and treatment courses and to elucidate molecular mechanisms driving tumor evolution and therapy resistance.

Methods
Intra-tumoral heterogeneity will first be assessed using our own patient cohort and datasets from the International Cancer Genome Consortium (ICGC). A longitudinal, multi-regional cohort of over 80 patients has been established to analyze tumor evolution across different sites and therapy stages. Bulk and single-cell genome and transcriptome sequencing of spatially and temporally matched tumor samples will be performed. Bioinformatic analyses will reconstruct clonal architectures and infer evolutionary trajectories within and between tumor regions.

Results
Bulk sequencing of individual tumor regions reveals significant genetic heterogeneity. Early analyses of matched pre- and post-treatment samples indicate branched evolutionary patterns. Expanding to a larger cohort and integrating single-cell data will enable reconstruction of a detailed temporal and spatial map of subclonal dynamics, outlining the evolutionary landscape of HNSCC.

Conclusion
The study is expected to uncover key molecular mechanisms underlying HNSCC progression and therapeutic resistance. Insights gained from tracking clonal dynamics may pave the way for novel strategies to develop and tailor patient-specific treatment approaches.

Poster Group 1

P07

Hsp70 as a Predictive Biomarker of Neoadjuvant Immunotherapy Response in HNSCC Revealed by Single-cell Analysis

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Background
Although immune checkpoint inhibitors (ICI) have introduced new therapeutic opportunities for head and neck squamous cell carcinoma (HNSCC), patient responses remain highly variable. Clinical trials are evaluating neoadjuvant immunotherapy for its potential to downstage tumors and preserve organ function, yet few biomarkers, most notably PD-L1, reliably predict benefit. In our prior proteomic analysis of platelet-derived exosomes from HNSCC and matched normal tissues, Hsp70 was identified as a hub protein, suggesting a role in tumor progression and microenvironment modulation. Building on this, we investigated whether Hsp70 expression is associated with response to immunotherapy.

Methods
In this hypothesis-generating study, we analyzed a GEO single-cell RNA sequencing dataset from a clinical trial where patients with HNSCC received neoadjuvant ICI therapy followed by surgery. After quality control, processing, and cell annotation, patients were classified as responders or non-responders. Comparisons were made between groups to assess cellular composition and differential gene expression.

Results
Cell type proportions showed no significant differences between responders and non-responders, indicating transcriptional changes were not driven by compositional bias. Notably, HSP70 expression was consistently downregulated in responders across nearly all cell types, ranking among the top 10 downregulated genes, with fibroblasts the only exception. In total, 1,993 genes were differentially expressed, including 1,070 upregulated and 923 downregulated in responders.

Conclusions
Our findings demonstrate Hsp70 downregulation is associated with a favorable response to neoadjuvant ICI therapy in HNSCC. This highlights Hsp70 as a potential predictive biomarker beyond PD-L1, with implications for patient stratification, treatment optimization, and therapeutic development. Further validation in larger, prospective cohorts is warranted to confirm clinical utility.

Fig. **1**

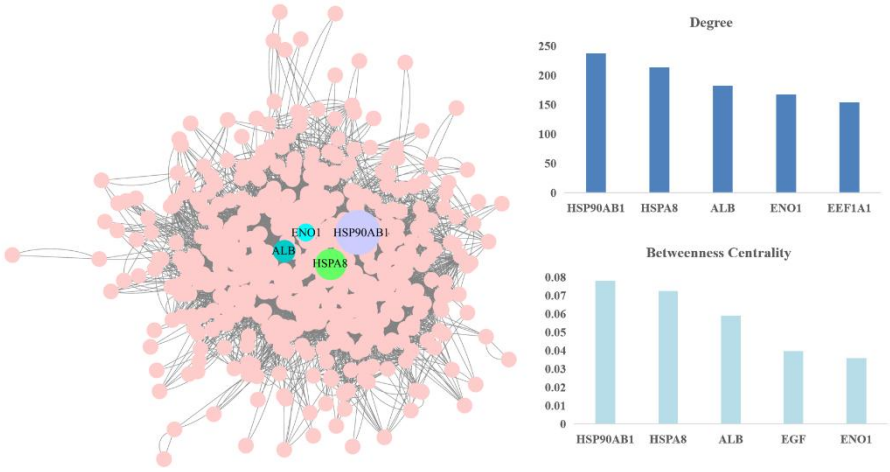


Fig. **2**

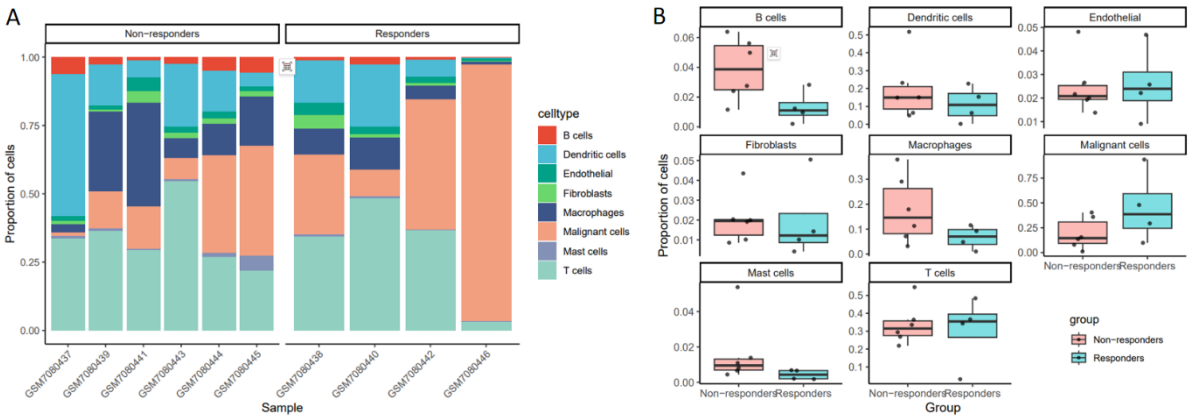
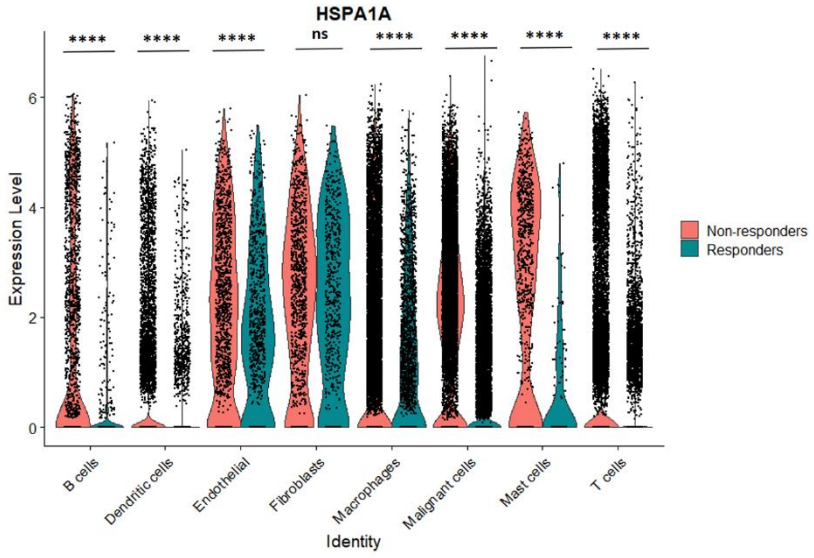


Fig. 3



Poster Group 1

P08

The German National Initiative for NeoAdjuvant Therapy in Head and Neck Cancer (NINA-KHT): Potential Biosamples and Cooperations

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Questions

The results of KN-689 study will mark a paradigm shift for therapy for locally advanced HNSCC integrating perioperative immunotherapy as a standard of care concept in future. This opens new opportunities for observational translational studies. Immunoprofiling of immunotherapy responders and non-responders could detect predictive marker for immunotherapy success in future.

What could be done with translational biobank samples collected in a German national noninterventional trial on neoadjuvant immune therapy in head and neck cancer?

What are chances of combining a robust clinical dataset and QoL database with translational biosamples?

Methods

We will present the current protocol for the clinical noninterventional trial and present potential biosamples and possibilities for cooperation with translational research groups. The timeline for the clinical trial is H1 2025 with start of biobanking in late H2 2025 to H1 2026.

Conclusion

The aim of this presentation is to discuss potential interaction points and encourage submission of translational projects within the upcoming biobank.

Poster Group 2

P09

Therapy-Induced Modulation of Tumor–Immune Crosstalk: Effects of Kinase Inhibition and Hypofractionated Radiotherapy in 3D HNSCC Models

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Question

HPV-negative head and neck squamous cell carcinomas (HNSCC) are still challenging due to their pronounced radioresistance. A promising approach to counteract this resistance is the use of DNA damage repair inhibitors (DDRi) in combination with radiotherapy (RT). To explore the resulting immunological responses, we established a 3D spheroid culture model. The tumor microenvironment was further mimicked by co-culturing human CD8+ T cells. This study aimed to assess immunomodulatory alterations in T cells following treatment of HNSCC spheroids with RT and DDRi.

Methods

HPV-neg. and HPV-pos. HNSCC cell lines were seeded in *low-attachment U-bottom* plates. After 24h, spheroids were treated with 1 µM AZD0156 (ATMi) or 0.1 µM VE-822 (ATRi), or in combination with hypofractionated RT (2 × 5 Gy). Also, CD8+ T cells (CTL) were isolated from healthy blood using MACS beads, labeled with CFSE, and CD3/CD28-stimulated for 48 hours. Flow cytometry was used to analyze the proliferation and expression of the activation markers on the T cells 48 h after co-cultivation. T cell migration was analyses using boyden chamber and immunhistochemical evaluation was done on spheroid slices in cooperation with the Pathology Erlangen.

Results

Our results revealed that HNSCC spheroids modulate CTL function depending on DDRi. ATRi with RT promoted a more favorable immune consequence, with enhanced CTL proliferation, migration, and spheroid infiltration, accompanied by reduced PD-L1 expression on tumor cells. In contrast, ATMi created a more immunosuppressive environment, impairing CTL activity and increasing PD-1/PD-L1 expression, thereby suggesting a rationale for combining ATMi + RT with immune checkpoint blockade.

Conclusions

Overall, ATRi proved more effective than ATM blockade in enhancing T cell activation. The limited immunostimulatory capacity of ATMi may reduce its clinical relevance, particularly as ATR inhibitors continue to show superior therapeutic potential.

Poster Group 2

P10

MAP-HNC: A Multi-Modal Tissue Analysis Platform to Uncover the Spatial Biology of Intratumoral Compartments in Head and Neck Cancer (HNC)

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Background
HNC features a complex tumor microenvironment (TME) with intratumoral heterogeneity and immune infiltration, complicating diagnosis and treatment. We developed MAP-HNC, an innovative spatial biology platform with cutting-edge multiple molecular modalities to characterize tumor spatial organization, integrated analysis of tissue architecture, gene/protein expression, immune cell marker profiling and metabolic states within the TME.

Methods
MAP-HNC analyzes consecutive tissue sections using:

1. histopathology (H&E staining)
2. spatial transcriptomics (RNAscope)
3. high-dimensional proteomics (MACSima)
4. spatial lipidomics (MALDI imaging mass spectrometry)
5. spatial metabolomics

Individual sections were scanned, aligned, classified and analyzed by HALO AI supported software.

Results
H&E staining along with cell nucleus detection of sequential HNC frozen tissue sections from oral cavity allowed alignment and identification of structural regions within the tumor. Multi-modal analysis revealed cytokine enriched regions that were characterized by high inflammatory immune cells and distinct metabolomic and lipidomic profiles. Application of tailored antibody panels suggested functional specialization and spatial segregation of immune cells along with the identification of distinct inflammatory, immunosuppressive versus T cell dominated, immune activating regions.

Perspective
MAP-HNC identifies complex biological features and spatial niches in HNC. Initial data point to strikingly distinct biological features of tumor parenchyma versus stroma along with the identification of spatially organized immune cell neighborhoods

Poster Group 2

P11

Cancer Associated Fibroblasts as a Predictor of Lymph Node Status and Extranodal Extension in Oropharyngeal Cancer

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Oropharyngeal cancer (OPC) presents a persistent challenge with a five-year survival rate of approximately 50%, which is largely attributed to occult metastasis and recurrence, particularly in cases involving lymph node positivity and extranodal extension (ENE). The presence of ENE represents an important risk and prognostic factor, which has recently been integrated into the staging algorithm for OPC. Recent advances in understanding the tumor microenvironment have highlighted the role of cancer-associated fibroblasts (CAFs) in cancer progression, with certain CAF markers showing potential as indicators of aggressive tumor behavior which can guide treatment planning. We aim to explore the relationship between specific CAF markers and key clinicopathological features, such as lymph node involvement and ENE, in OPC. We analyzed 30 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from transoral robotic surgery (TORS) resected OPC. These samples were categorized into three pathological subgroups: node-negative/ENE-negative, node-positive/ENE-negative, and node-positive/ENE-positive, matched for demographic variables including age, sex, and smoking history. Immunohistochemical (IHC) staining was performed using markers for CAFs (IL6, CD29, PDPN, FAP), epithelial cells (PanCK), and immune cells (CD45). The analysis was conducted using QuPath software, which facilitated nucleus segmentation and the application of a machine learning classifier for intensity detection. Preliminary findings suggest an association between the expression of CAF markers and more aggressive tumor features, which offers promising insights into the role of CAFs in OPC progression and thereby enhancing treatment planning in OPC.

Poster Group 2

P12

Investigating the Impact of EGFR Signaling on Response to Nivolumab in Head and Neck Squamous Cell Carcinoma

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Question

Clinical responses to anti–PD-1 therapy in head and neck squamous cell carcinoma (HNSCC) remain limited, and the mechanisms underlying resistance are not fully understood. Epidermal growth factor receptor (EGFR) signaling is frequently activated in HNSCC and may contribute to an immunosuppressive tumor phenotype. This study investigated how EGFR pathway activation influences tumor immunogenicity and response to PD-1 blockade.

Methods

Transcriptomic data from a HNSCC cell line model following EGF treatment were analyzed to assess the immunological consequences of EGFR activation. Additionally, publicly available single-cell and bulk RNA sequencing data from patients with locally advanced HNSCC treated with neoadjuvant Nivolumab were analyzed. Cell subtypes within the tumor microenvironment were defined, and their relative abundances were correlated with treatment response.

Results

EGF treatment induced upregulation of immunosuppressive molecules, including PD-L1, accompanied by downregulation of genes involved in antigen processing and presentation. In patient samples, a tumor cell subtype characterized by high EGFR signaling was associated with inferior treatment response and showed relative enrichment post-treatment, suggesting a link between EGFR activity and immune resistance.

Conclusion

EGFR signaling promotes an immunosuppressive tumor phenotype in HNSCC and may contribute to limited responsiveness to PD-1 blockade. Combined targeting of EGFR and PD-1 pathways could enhance antitumor immunity and improve clinical outcomes.

Poster Group 2

P13

Interleukin Expression in Patient-derived Head and Neck Cancer Models Cultured in 2D and 3D

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Objective

Members of the interleukin (IL) family are known to exert both pro-tumorigenic (e.g., IL-1, IL-8) and anti-tumorigenic (e.g., IL-15, IL-24) effects in head and neck cancer (HNC). Some ILs are directly expressed by tumor cells. Since several ILs are considered potentially druggable targets, appropriate experimental models are required to assess their functions. This study aimed to evaluate whether patient-derived HNC cells cultured in 2D and 3D conditions are suitable models for studying IL expression and potential IL-mediated tumor cell effects.

Methods

Tumor samples from HNC patients (n = 8) were analyzed by single-cell RNA sequencing to identify IL gene expression profiles. Patient-derived tumor cells (n = 3, ethics approval no. 2018-603N-MA) were isolated using either outgrowth culture (OC) or enzymatic digestion (ED) and expanded in 2D cultures up to passage 2. These cells were used to form 3D spheroids (25,000 cells/spheroid, cultured for 7 days) or isolate total RNA (Isolate II RNA Mini Kit). The isolated mRNA was analyzed using the nCounter platform (Bruker/NanoString) and a cancer gene panel including 32 IL genes. Data analysis was performed using n-Solver Analysis Software 4.0.

Results

Single-cell RNA sequencing identified expression of 11 IL genes (including IL-1, IL-6, IL-15) in patient tumor samples. In cultured cells, reproducible expression was detected for 6 ILs. Certain cytokines showed isolation- or culture-dependent expression: IL-11 was observed only in ED-derived cells, IL-19 only in 3D spheroids, while IL-1α and IL-1β were more strongly expressed in 2D cultures. IL-15 was expressed equally in all cell culture samples. IL-8 was detected in cultured cells but not in freshly isolated cells.

Conclusion

Not all ILs expressed in primary tumor tissues are retained in cell culture, and some ILs appear only under in vitro conditions. Therefore, IL expression should be verified in HNC models before using them for functional studies.

Poster Group 2

P14

Irradiation Triggers IL-8-Mediated Neutrophil Infiltration in Human Head and Neck Squamous Cell Carcinom

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Neutrophils are immune cells involved in innate immunity that play a role in many aspects of tumor growth and metastasis. The neutrophil-to-lymphocyte (NLR) ratio is very important for treating patients with head and neck squamous cell carcinoma (HNSCC), and high levels of tumor-infiltrating neutrophils, which support tumor growth, are linked to poor prognosis. Data on mediators of neutrophil migration and the effects of standard treatments like radiotherapy (RT) are not yet well understood.

We cultured HNSCC cell lines and exposed them to fractionated ionizing radiation. The secretomes of these tumor cells and their direct effects on native neutrophil phenotype, as well as neutrophil migration toward these tumor secretomes, were analyzed using bead-based chemokine measurements, transwell migration assays, and flow cytometry. We identified IL-8 as a key regulator of neutrophil migration. To evaluate its role, IL-8 was silenced in the HNSCC cell line FaDu with siRNA, and its impact on neutrophil migration and phenotype was assessed through transwell assays and mRNA sequencing.

We found that RT influenced IL-8 secretion in certain cell lines. Higher IL-8 levels in the secretomes were associated with increased inflammatory responses from neutrophils and greater neutrophil migration toward these secretomes. Silencing IL-8 reduced this response, and mRNA sequencing showed that, without IL-8 in the secretomes, autocrine IL-8 signaling in neutrophils was decreased. Furthermore, IL-8 silencing resulted in lower levels of neutrophil migration markers like CD177 and CD62L.

Our findings indicate that irradiating HNSCC tumor cells triggers IL-8-driven neutrophil infiltration into the tumor. This process may be aided by the neutrophil-specific migration molecule CD177, presenting a potential target for combined radiotherapy in HNSCC. The strategy is designed to limit excessive neutrophil invasion, which is associated with a worse prognosis.

Poster Group 2

P15

Platelet-Driven Remodeling of the Tumor Microenvironment in 3D Organoids of HNSCC

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Objective

Head and neck squamous cell carcinoma (HNSCC) is a highly lethal malignancy shaped by a complex tumor microenvironment (TME), where platelets play pivotal roles in driving tumor progression, immune evasion, and therapeutic resistance. Conventional 2D models fail to capture these interactions, underscoring the need for advanced systems that recapitulate tumor–host dynamics.

Method

We established a patient-derived 3D organoid model to investigate how platelets remodel the TME. Organoids were exposed to donor-derived platelets, followed by metabolomic profiling and functional assays.

Results

Necmarkers (ceramides, sphingomyelins, cholesterol, phosphatidylethanolamines) increased markedly in platelet-exposed organoids, consistent with accelerated cell death and tissue damage. TME/tumor-stroma markers (phospholipids, carnitines, fatty acids) also increased under platelet influence, indicating enhanced lipid turnover, membrane remodeling, and energy metabolism fueling invasion and stromal interactions. Collectively, these shifts highlight platelet-driven metabolic reprogramming as a mechanism that promotes necrosis, invasion, and stromal remodeling. Histology and imaging supported these findings: hematoxylin staining revealed greater invasion into dermal equivalents, while raster-scanning optoacoustic mesoscopy (RSOM) confirmed significant platelet-driven vascularization.

Poster Group 2

P16
Characterization of NPC Killing by NK Cells via IFNβ

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Background
Nasopharyngeal carcinoma (NPC) is a highly aggressive epithelial tumor strongly associated with Epstein-Barr virus (EBV) infection. In pediatric and adolescent patients, the addition of interferon-β (IFNβ) to standard chemo- and radiochemotherapy has significantly improved survival rates, exceeding 90%. Previous studies demonstrated that IFNβ-activated natural killer (NK) cells selectively utilize TRAIL to eliminate NPC cells. This interaction leads to the release of soluble TRAIL and downregulation of membrane-bound TRAIL. The present study aims to elucidate the mechanisms by which NPC cells selectively activate NK cells for TRAIL-mediated cytotoxicity and to identify the pathways involved in the secretion of soluble TRAIL by IFNβ-activated NK cells.

Methods
Using the NPC cell line HK1, we analyzed canonical cytotoxic pathways (TRAIL, FasL, perforin/granzyme B) in IFNβ-activated NK cells via flow cytometry. Selectively, TRAIL-mediated cytotoxicity was further investigated using ELISA, siRNA knockdown, and pharmacological inhibition. The requirement for direct cell-cell contact versus indirect signaling was assessed using calcein-release assays and Transwell co-culture systems.

Results
Cytotoxicity assays confirmed that NK cells primarily kill NPC cells via the TRAIL pathway. Both direct contact and soluble factors contributed to TRAIL induction and NPC cell death. IFNβ significantly enhanced TRAIL expression in NK and NPC cells in a time-dependent manner, increasing NK cell-mediated cytotoxicity. Inhibition of the protease ADAM17 modulated surface TRAIL expression and altered soluble TRAIL levels in NK:NPC co-cultures, suggesting a regulatory role of ADAM17 in IFNβ-induced TRAIL secretion.

Conclusion
These findings provide novel insights into the regulation of TRAIL by IFNβ and suggest that targeting TRAIL pathways may represent a promising immunotherapeutic strategy for the treatment of NPC.

Poster Group 3

P17
SOX2-Driven Modulation of Immune Phenotypes in Head and Neck Squamous Cell Carcinoma

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SOX2 is a frequently amplified gene in head and neck squamous cell carcinoma (HNSC). However, low SOX2 expression has been associated with increased migration, invasion, as well as poor prognosis. Nevertheless, the role of SOX2 in immune evasion in HNSC remains poorly understood.To characterize the immune landscape associated with SOX2 expression and to elucidate its potential role in shaping antitumor immune responses in HNSC.We utilized bulk-RNA sequencing data from TCGA-HNSC as a training cohort and validated key findings in independent cohorts (CPTAC-HNSC, GSE65858, GSE275870, GSE117973) as well as TCGA pan-SCC datasets (TCGA-LUSC, TCGA-CESC, TCGA-ESCA). The bioinformatics workflows included gene set variation analysis (GSVA) of hallmark gene sets, deconvolution analysis, such as CIBERSORT, xCell, MCP-counter, quantseq and Kassandra of immune cell subsets, and the analysis of ligand–receptor pairs with BulkSignalR. An exploratory analysis was conducted using HNSC cell lines in vitro in order to validate the predicted models.GSVA analysis revealed an inverse correlation between SOX2 expression and gene sets associated with immune response (e.g. interferon signaling, antigen presentation machinery, inflammatory response) in both HNSC and pan-SCC cohorts. Immune cell deconvolution revealed that tumors with low SOX2 expression exhibited higher infiltration of B cells and $CD4^+$ T helper cells. These tumors were also characterized by ligand–receptor pairs indicating enhanced immune-related cell communication. Mechanistically, SOX2 expression in tumor cells was inversely correlated with interferon-induced STAT1 activity and PD-L1 expression.These data suggest that there are different modes of immune escape in SCC dependent on SOX2 expression. While tumors with low SOX2 expression are more likely to respond to immune checkpoint inhibition (ICI), targeting the SOX2–JAK–STAT1–axis may provide an alternative therapeutic approach for tumors with high SOX2 expression.

Poster Group 3

P18

Functional Characterization of LYVE-1+ Macrophages in the Tumor-microenvironment of Head and Neck Squamous Cell Carcinoma

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Objective
Head and neck squamous cell carcinoma (HNSCC) are characterized by a dense immune infiltrate, including macrophages (MΦ) (Peltanova et al. 2019). In HNSCC tissues we identified LYVE-1+ MΦ, a distinct subset closely associated with vasculature and stromal remodeling. While implicated in extracellular matrix remodeling, immune modulation, and angiogenesis in other cancers (Dollt et al. 2017; Etzerodt et al. 2020; Strack et al. 2020; Nalio Ramos et al. 2022), their role in HNSCC remains unclear.

Methods
Multiplex immunohistochemistry was used to assess spatial distribution of LYVE-1+ MΦ. Human monocytes were differentiated into LYVE-1+ MΦ in vitro using M-CSF, dexamethasone, and IL-4 (MDI). Monocyte-derived LYVE-1+ MΦ (MDI-MΦ) were analyzed by flow cytometry, Western blot and qPCR. Functional assays included phagocytosis, T-cell activation, fibroblast proliferation, lymphangiogenesis and tumor colony formation.

Results
LYVE-1+ MΦ were enriched near lymphatic endothelial cells, vessels, and fibroblasts in non-metastatic HNSCC, but markedly reduced in metastatic tumors. In vitro, MDI-MΦ displayed an M2-like phenotype with increased CD163, CD206, and MerTK expression. They showed enhanced efferocytosis of apoptotic cells. Conditioned supernatants significantly inhibited fibroblast proliferation, contrasting with other macrophage subsets. In colony assays, MDI-MΦ supernatants suppressed tumor growth in one HNSCC cell line but showed no effect in another. LYVE-1+ MΦ had no major effect on T-cell activation or lymphangiogenesis.

Conclusion
LYVE-1+ MΦ play a complex role in HNSCC by influencing stromal remodeling, immune modulation, and tumor cell behavior. Their enhanced efferocytosis, inhibitory effect on fibroblast proliferation and selective suppression of tumor colony formation suggest a unique regulatory function within the tumor-microenvironment. These findings identify LYVE-1+ MΦ as potential therapeutic targets warranting further investigation.

Poster Group 3

P19

The Interaction of BTK and NFATc2 and its Impact on Head and Neck Squamous Cell Carcinoma (HNSCC)

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Question

HNSCC is the sixth most prevalent cancer worldwide. Despite the advancements in treatment over the last years, the prognosis remains poor. A variety of molecular markers and pathways have been identified, that play a crucial role in tumorigenesis, progression and metastasis in HNSCC. The present study investigated the effect of Bruton"s tyrosine kinase (BTK) and nuclear factor of activated t-cells (NFATc2) in HNSCC and the interaction between these two molecules including the potential downstream pathways.

Methods

Human HNSCC cell lines were cultivated in 2D and 3D cell culture. The expression level of NFATc2 was analyzed in a Western Blot. 3D cell cultures were treated with the BTK-inhibitor AVL-292 (10µM, 20 µM) and effects on NFATc2 expression levels and downstream pathways, including epithelial-mesenchymal transition and the p53 pathway were analyzed. Subsequently, NFATc2-knockdown was performed with siRNA, followed by downstream pathway analysis and functional assays, including proliferation and migration assays, an apoptosis assay, and cell cycle analysis in 2D cell cultures.

Results

The existence of NFATc2-positive and -negative HNSCC cell lines has been observed. BTK inhibition demonstrated a significant decrease of NFATc2 expression and showed a substantial impact on certain EMT-markers in specific HNSCC cell lines, while the knockdown of NFATc2 exhibited no notable effect. In functional assays, the knockdown of NFATc2 significantly impacts cell proliferation and migration, while having no effect on cell cycle or apoptosis.

Conclusions

NFATc2 expression is heterogeneous among HNSCC cell lines and can be downregulated by BTK inhibition. NFATc2 knockdown reduces cell proliferation and migration, whereas BTK inhibition influences EMT-related pathways. These data suggest that BTK and NFATc2 are functionally linked but may act through distinct downstream mechanisms. These findings underscore the heterogeneity in molecular patterns of HNSCC. Further studies are required to clarify their role in HNSCC.

Poster Group 3

P20
Modulation of T Cells by Myeloid Cells in Head and Neck Cancer

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Head and neck cancer (HNC), of which over 90% are squamous cell carcinomas (SCC), presents a highly inflamed tumor microenvironment rich in tumor-infiltrating leukocytes. The distinct separation between tumor parenchyma and mesenchymal stroma makes HNC a typical cancer type in the study of cell behavior in the two principal spatial compartments of primary carcinoma lesions. In this study we analyzed the spatial interaction of main myeloid immune cell types and T cell subsets in tumor parenchyma and stroma of head and neck SCC (HNSCC).

To this end, we used multiplex immunofluorescence staining and spatial analysis to quantify neutrophils, macrophages, and $CD8^+$ T cells in 14 HNSCC patient samples. We also examined correlations between myeloid cell densities and $CD8^+$ T cells, and assessed contact-dependent suppression of $CD8^+$ T cell proliferation.

When comparing the two intratumoral compartments we found that the tumor parenchyma contained significantly fewer neutrophils, macrophages, and $CD8^+$ T cells than the mesenchymal stroma. However, $CD8^+$ T cell proliferation was higher in the tumor parenchyma than in the stroma. In the stroma, $CD8^+$ T cell density negatively correlated with neutrophil density and positively with macrophage density per patient. Spatial analysis revealed that macrophages co-localized with both neutrophils and $CD8^+$ T cells in both compartments, whereas neutrophils spatially excluded $CD8^+$ T cells in the stroma. Functionally, neutrophils suppressed $CD8^+$ T cell proliferation via a contact-dependent mechanism. This study indicates divergent roles for macrophages and neutrophils in the tumor microenvironment of HNSCC.

Poster Group 3

P21

Critical Role of IFNAR1 Signaling in Neutrophil-Mediated Antitumor Immunity and CD8 T Cell Activation in Head and Neck Cancer

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This study investigates the immunoregulatory role of neutrophils in the tumor microenvironment of head and neck squamous cell carcinoma, highlighting the critical plasticity of neutrophil subsets influenced by type I interferon signaling. Neutrophils can adopt either pro-tumoral or anti-tumoral phenotypes, largely determined by signals such as IFNAR1-mediated pathways which orchestrate cytotoxicity, antigen presentation, and immune surveillance.

IFNAR1 deficiency in HNC increased tumor burden and worsened survival by promoting neutrophil infiltration with a pro-tumoral phenotype. Immunohistochemical analysis of patient samples further revealed significantly reduced IFNAR1 expression in tumor tissues from patients with poor prognosis compared to non-tumor controls, emphasizing its clinical relevance. Single-cell transcriptomic profiling demonstrated that IFNAR1 signaling maintains critical neutrophil diversity by sustaining subsets characterized by robust interferon-stimulated gene expression, including antigen presentation and proinflammatory pathways. Functional assays using HoxB8 cells showed that intact IFNAR1 signaling promotes CD8 T cell activation and enhances tumor cell killing. Additionally, CD8 T cells isolated from tumors of WT mice exhibited higher levels of Granzyme B and perforin compared to those from Ifnar1-/-, indicative of enhanced cytotoxic potential. CD8 T cells from tumor-draining lymph nodes in WT mice also showed increased proliferation, marked by elevated Ki67 expression, underscoring a more robust antitumor T cell response linked to intact IFNAR1 signaling.

The IFNAR1 signaling axis is essential for regulating neutrophil heterogeneity and promoting antitumor immunity. Restoration of IFNAR1 function in neutrophils may represent a promising strategy to reactivate immune surveillance and improve therapeutic outcomes in cancer patients.

Poster Group 3

P22

Protective Structures Compromised: Neutrophil Enrichment within TLS Drives Poor Survival in Head and Neck Cancer

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Question

Neutrophils modulate the tumor microenvironment; however, their role in tertiary lymphoid structures (TLS) in head and neck squamous cell carcinoma (HNSCC) remains unclear. TLS have been associated with improved survival, which suggests that neutrophils influence TLS function and patient outcomes. The aim of this study is to characterize neutrophil phenotypes in saliva, blood, and tumor tissue and relate them to TLS biology and survival.

Methods

HNSCC patients were analyzed using multiparameter flow cytometry on saliva samples and multiplex immunofluorescence (IF) on matched tumor sections. The panels included markers of neutrophil activation. TLS status was assessed using CD19/CD3 IF. Previously stained TLS-positive/-negative tumors were reanalyzed for NET markers and chemokine receptors. Ongoing work includes paired FACS of blood and tumor single-cell suspensions with extended neutrophil, T/B-cell activation.

Results

Quantitative analyses revealed that a higher proportion of neutrophils relative to B- and T-cells within TLSs correlated with reduced overall survival, suggesting that an imbalanced cellular composition has an impact. FACS revealed that CXCR4 expression was higher in oral neutrophils from TLS-negative patients ($p<0.05$). NET analyses and paired blood/tumor cytometry are ongoing.

Conclusions

Distinct neutrophil phenotypes, particularly CXCR4 expression and relative TLS abundance, are associated with TLS status and survival in HNSCC. Although TLSs generally predict a favorable outcome, a high neutrophil-to-lymphocyte ratio within TLSs appears to impair their protective function. Combined FACS and histology provide a framework to unravel these interactions and may have an impact on predictive/therapeutic strategies in HNSCC.

Poster Group 3

P23

SOX2 Modulates Alcohol-Induced Epithelial-Mesenchymal Transition and Predicts Divergent Prognosis in Oral Squamous Cell Carcinoma

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Background
Oral squamous cell carcinoma (OSCC), the most common subtype of head and neck cancer, is an invasive malignant tumor with a five-year survival rate below 50%. Alcohol relates to tumor progression and poor prognosis via malignant behaviors, such as metastasis, through mechanisms including the induction of epithelial-to-mesenchymal transition (EMT). SOX2, a transcription factor frequently amplified in OSCC, has been shown to suppress EMT and tumor cell dissemination in a context-dependent manner. We hypothesize that alcohol drives OSCC progression by inducing EMT in a SOX2-dependent manner.

Methods
Multi-omics and clinical data were collected from the TCGA-OSCC (n = 301), GSE65858 (n = 83), and CPTAC-OSCC (n = 43) cohorts, and the expression of EMT-related gene sets was evaluated using Gene Set Variation Analysis (GSVA). Kaplan-Meier survival analysis was performed to assess progression-free survival (PFS) in alcohol-stratified subgroups and based on SOX2 expression. Ethanol was applied to assess morphological and molecular EMT markers in OSCC cell lines in vitro by immunofluorescence.

Results
EMT pathway activity was upregulated in OSCC patients with an alcohol consumption history across all cohorts. Among alcohol consumers, high SOX2 expression was inversely correlated with EMT GSVA scores and was significantly associated with improved PFS. However, no significant association was observed between SOX2 and EMT or survival in OSCC patients without alcohol consumption. In vitro, ethanol treatment induced mesenchymal-like morphology and vimentin expression - well-established EMT markers - in OSCC cells in a SOX2-dependent manner.

Conclusions
This study confirms that ethanol promotes EMT in OSCC and provides experimental evidence for the context-specific role of SOX2. The presented data provide new insights into the risk stratification of alcohol-associated OSCC and may pave the way for innovative concepts of targeted and personalized treatment.

Poster Group 4

P24

Multifaceted Anti-Cancer Potential of 1,8-Cineole in HNSCC: Targeting Proliferation, Metabolism, and Apoptosis Pathways

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Head and neck squamous cell carcinoma (HNSCC) is a highly aggressive malignancy with stagnant survival outcomes, driven by recurrence, therapy resistance, and tumor-promoting inflammation. Conventional treatments typically inhibit single pathways while leaving alternative survival signals, metabolic reprogramming, and inflammatory interactions intact. 1,8-Cineole, a clinically approved monoterpene with established anti-inflammatory, antioxidant, and anti-proliferative properties, emerges as a promising candidate to disrupt both tumor-intrinsic and microenvironmental resistance mechanisms. Building on its favorable pharmacological profile and clinical safety, we investigated its anti-tumor potential in HNSCC cell models.

HNSCC cells were treated with cineole. Proliferation was assessed by EdU assay and motility was analyzed using scratch assay. Alterations in signaling pathways were monitored by proteomic analysis. Platelet–leukocyte interactions were examined with binding affinity assay.

Cineole treatment reduced proliferation, impaired motility, and promoted apoptosis in HNSCC cells. Mechanistically, cineole activated p38 MAPK while downregulating ERK, AKT, and NF-κB, linking stress signaling to reduced survival and inflammatory responses. Enhanced GSK-3β activity further attenuated Wnt/β-catenin-driven proliferation and metabolic adaptation. Importantly, cineole disrupted platelet–leukocyte aggregates via P-selectin suppression, underscoring its ability to remodel the inflammatory tumor microenvironment in addition to direct tumor cell targeting.

These findings establish cineole as a multifaceted anti-cancer agent in HNSCC. Its dual action on tumor cells and the microenvironment positions it as a translational strategy to overcome resistance and improve outcomes. Given its availability, safety, and pleiotropic effects, cineole holds promise for integration into HNSCC therapy. Ongoing in vivo studies aim to validate these effects and support clinical translation.

Poster Group 4

P25

Preliminary miRNA Profiling in ERp29-Silenced Tongue Squamous Cell Carcinoma Highlights Cancer-Related Pathways

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Objective
Endoplasmic reticulum protein 29 (Erp29) has been implicated in tumor biology, but its role in tongue squamous cell carcinoma (TSCC) remains unclear. We aimed to identify microRNA (miRNA) alterations and downstream pathways triggered by ERp29 silencing.

Methods
SCC-4 TSCC cells (ATCC, CRL-1624) were edited with CRISPR/Cas9 to knockout *ERP29*. miRNA expression was profiled using nCounter® miRNA Expression Assay Kit (NanoString®), normalized to counts-per-million, and analyzed for differential expression versus parental SCC-4. Validated miRNA targets were retrieved from the multiMiR database and subjected to KEGG and Reactome enrichment analyses.

Results
ERp29 loss was associated with down-regulation of several miRNAs, including hsa-miR-27b-3p, hsa-miR-30a-5p, hsa-miR-19b-3p, hsa-miR-320e, hsa-miR-378i, hsa-miR-126-3p, has-miR-1306-5p, and has-miR-616-3p (absolute $\log_2$ fold-change > 8). KEGG analysis revealed strong enrichment of proteoglycans in cancer (hsa05205) and PI3K/AKT signaling (hsa04151), encompassing canonical TSCC drivers such as *TP53* (Entrez ID 7157), *PIK3CA* (ID 5290), *EGFR* (ID 1956), *HRAS* (ID 3265), *AKT1* (ID 207), *PTEN* (ID 5728), and *MAPK1/3* (IDs 5594/5595). Reactome enrichment highlighted pathways such as signaling by ALK in cancer (HSA-9700206), NOTCH1 signaling (HSA-2644603), and AKT1 E17K activation (HSA-5674400), all related to growth-factor signaling, extracellular matrix remodeling, and immune crosstalk.

Conclusion
These pilot findings, derived from a single TSCC cell line, indicate that Erp29 loss could reshapes the miRNA landscape and enriches gene networks linked to PI3K/AKT, NOTCH, and proteoglycan-mediated cancer progression. The results are preliminary and require validation in additional cell lines and clinical samples to confirm the role of Erp29 in tumor-host interactions. Financial support: CNPq #408177/2023-3 and #307944/2022-0, FAPESP CancerThera/CEPID #2021/10265-8, and CAPES.

Poster Group 4

P26

Impact of *PDCD1* Variants on Risk, Clinicopathological Aspects and Prognosis in Patients with Laryngeal Squamous Cell Carcinoma

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Objective
Laryngeal squamous cell carcinoma (LSCC) development and progression depend on immune evasion. The PD-1/PD-L1 pathway is one of the main mechanisms of immune escape, since interaction within this system leads to T lymphocyte proliferation or apoptosis. The *PDCD1* gene encodes the PD-1 protein and is polymorphic. Thus, this study aimed to evaluate the influence of PD1 (rs41386349, C>T), PD1.1 (rs36084323, G>A), PD1.5 (rs2227981, C>T), and PD1.9 (rs2227982, C>T) single nucleotide variants (SNVs) in the *PDCD1* gene on the risk, clinicopathological aspects, and overall survival (OS) and event-free survival (EFS) of patients with LSCC.

Methods
This study enrolled 286 LSCC patients and 296 controls (blood donors). *PDCD1* genotypes were identified by real-time PCR. Kaplan-Meier curve, log-rank test, and univariate and multivariate Cox analyses were used to evaluate the impact of clinicopathological and genetic factors in survival of HNSCC patients.

Results
Similar frequencies of *PDCD1* genotypes were observed in patients and controls. An excess of CC genotype of PD1 was seen in patients with stage III or IV tumors. GA or AA + CT or TT (PD1.1 + PD1.5) and CT or TT + CT or TT (PD1.5 + PD1.9) combined genotypes were more frequent in patients with glottic tumors. In multivariate analysis, patients with TT genotype of PD1.5 and CC + TT combined genotype of PD1 + PD1.5 had, respectively, 1.59- and 2.97-fold increased risk of death.

Conclusions
Our data presents preliminary evidence that 1) *PDCD1* SNVs do not influence LSCC risk, 2) PD1 influences LSCC aggressiveness, 3) PD1.1, PD1.5, and PD1.9 influence LSCC location, and 4) PD1 and PD1.5 are independent prognostic factors for overall survival in LSCC patients. Acknowledgments: The study was supported by FAPESP # 2019/09168-8, Cancer Theranostics Innovation Center # 2021/10265-8, and CNPq # 302922/2025-3.

Poster Group 4

P27

Modulation of PI3K/AKT Pathway Gene Expression by ERp29 Silencing in Cisplatin-Sensitive and -Resistant Pharyngeal Carcinoma Cells

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Objective

ERp29, a chaperone involved in protein folding and secretion, may affect tumor progression when dysregulated. *ERP29* silencing promotes pharyngeal carcinoma (PC) cell progression, suggesting a suppressive role in aggressive phenotypes, however, the mechanisms remain unclear. PI3K/AKT regulates tumor survival, but its modulation by *ERP29* is unknown. This study evaluated PI3K/AKT pathway gene expression in PC cells with *ERP29* suppression: untreated (FaDu), cisplatin-treated (FaDu-CDDP), and CDDP-resistant (FaDu-R).

Methods

FaDu cells were cultured and exposed to CDDP to induce resistance. *ERP29* was silenced using siRNA. PI3K/AKT pathway gene expression was assessed using the TaqMan Array Human Molecular Mechanisms of Cancer panel and validated by qPCR. Results were analyzed by *t*-test and expressed as fold change (FC). *p*-value <0.05 was significant.

Results

SRC expression was higher in FaDu-CDDP *vs* FaDu (FC: 3.4, *p*=0.02) and FaDu-R (FC: 4.6, *p*<0.001), equalizing after *ERP29* silencing. *AKT1* was elevated in FaDu (FC: 4.2, *p*=0.03) and FaDu-CDDP (FC: 3.9, *p*=0.04) *vs* FaDu-R; after silencing, higher in FaDu *vs* FaDu-CDDP (FC: 1.7, *p*=0.04). *ITGAV* showed no differences initially but increased in FaDu (FC: 3.3, *p*=0.02) and FaDu-R (FC: 2.3, *p*=0.01) *vs* FaDu-CDDP after silencing. *JUN* was higher in FaDu-CDDP *vs* FaDu-R (FC: 2.6, *p*=0.04); after *ERP29* silencing, higher in FaDu *vs* FaDu_CDDP (FC: 4.5, *p*=0.03) and *vs* FaDu-R (FC: 3.0, *p*=0.03). MDM2 was lower in FaDu *vs* FaDu-CDDP (FC: 0.3, *p*=0.002) and *vs* FaDu-R (FC: 0.2, *p*=0.01), but increased in FaDu vs FaDu-CDDP after silencing (FC: 2.0, *p*=0.02).

Conclusions

ERP29 modulates PI3K/AKT pathway genes, potentially affecting tumor behavior and CDDP resistance in PC. Strategies to restore *ERP29* function may represent novel treatment approaches. Further studies are needed to validate these findings. Financial support: CNPq (#408177/2023-3 and #307944/2022-0).

Poster Group 4

P28

Type-I Interferon-Neutrophil Crosstalk - The Tagteam Fighting Head and Neck Cancer-

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Type I interferons (IFNs) have a strong impact on anti-tumoral immune responses by modulating the function of various immune cells, including neutrophils. Tumors have shown to have mechanisms to evade immune control, such as downregulating the IFN signaling pathway.

However, the role of the IFNAR pathway, in head and neck cancer (HNC) and its immunological consequences, are still to be better understood.

In this study, we aimed to investigate the impact of IFNAR loss in HNC tumors, with a particular focus on tumor-associated neutrophils (TANs), their prognostic impact and potentially being the key to better outcomes in HPV positive tumors.

We assessed IFNAR expression via multicolor immunohistochemistry in tumor tissue of HNC patients, including HPV positive and negative. We correlated these findings with clinical outcomes, including 5-year survival data and further solidified our findings in a transplantable murine HNC model, comparing wild-type (IFNAR-sufficient) and IFNAR-deficient animals.

We were able to show significantly reduced IFNAR expression on neutrophils within tumor tissue in patients with poor prognosis. The murine model further showed, that IFNAR expression on neutrophils declined as tumors progressed. Preliminary transcriptomic data suggests the emergence of an IFN-associated signature linked to HNC survival.

Ongoing unbiased computational analyses will help us to further understand the relationship between IFN signaling in TANs and patient survival, potentially establishing IFNAR expression as a prognostic biomarker and therapeutic target in HNC.

Poster Group 4

P29

Tumor–Stroma Crosstalk: Cytokine Cues Shape Neutrophil Landscapes in Head and Neck CancerFrom Mechanistic Discovery to Clinical Relevance

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In head and neck cancer (HNC), high tumor-associated neutrophil (TAN) density is one of the most robust immunological biomarkers of poor outcome. To explore its mechanistic basis, we investigated how tumor-stroma crosstalk influences TAN distribution and plasticity. In order to identify mechanisms of TAN recruitment, we first screened primary mesenchymal stromal cells (MSC) for production of CXCL8 and G-CSF, two factors that direct neutrophil chemotaxis and survival. We found that stimulation of MSC with IL-1α, which can either be released from necrotic tumor cells or secreted from living cells, strongly induced production of both factors. Stimulated MSC differentiated into inflammatory carcinoma-associated fibroblasts (iCAF). The IL-1α–iCAF gene signature resembles CAF populations in HNC associated with immunotherapy response and survival. Mechanistically, iCAF-conditioned medium enhanced PMN survival, chemotaxis, and infiltration into IL-1α-stimulated MSC spheroids. Tumor spheroids exposed to IL-1α–iCAF signals showed accelerated growth, suggesting a stromal amplification loop that promotes neutrophil recruitment and tumor progression.

In HNC tissues, tumor IL1A expression strongly correlated with stromal co-expression of CXCL8 and CSF3, particularly in parenchyme-rich tumors with a high tumor-stroma ratio. Patients high in stromal double positive cells showed enhanced TAN density, especially in the tumor compartment, and an altered TAN marker profile. Spatial mapping showed enrichment of double-positive cells near areas of cell death and IL1A-high tumor–stroma interfaces.

These findings demonstrate that tumor cues initiate stromal activation, inducing cytokine networks that drive clinically relevant TAN recruitment and activation.

Poster Group 4

P30
Regulation of Laminin-332 Invasion on EGFR MAPK Axis and its Potential Inhibitors

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Objective
Laminin-332 is an extracellular matrix protein composed of α3, β3, and γ2 chains encoded by LAMA3, LAMB3, and LAMC2. The expression of this protein rises with EGF treatment over time. Thus, it can be hypothesized that Laminin-332 plays a role in EMT of HNSCC cells. We aim to find out the molecular signaling pathway and regulatory mechanisms of Laminin-332.

Methods
Producing a LAMC2- Knock-out cell line with CRISPR/Cas9 in HNSCC cell lines.

Using RT-qPCR, the gene expression of Laminin-332 is examined in cells treated with EGF and MAPK inhibitors.

3D invasion models FaDu WT and KO-ITGB4 cells are formed as spheroids and are embedded in a collagenase mix, which mimics the extracellular matrix. They are then be treated with EGF, Laminin-332, and inhibitors.

Results
To select effective inhibitors of EGFR signaling pathways, three were tested: Avutometinib (Raf/MEK inhibitor), Defactinib (FAK inhibitor), AZD0364 (ERK inhibitor). For each inhibitor cell viability experiments and 3D invasion models were performed. Avutometinib shows the optimal concentration at 25nM, Defactinib"s optimal concentration is at 500nM and AZD0364 at 10nM. These inhibitors were applied individually and in combination to assess the effects against the EGF-induced invasion. Avutometinib exhibited the best inhibitory effect, even better than Cetuximab, while Defactinib has shown a limited reduction in invasion. To further understand the impact of pathway inhibition at the transcriptional level, cells were treated with EGF and together with inhibitors Cetuximab, MEK-inhibitor and Avutometinib. All three inhibitors reduced the expression of LAMC2, with MEK inhibition and Avutometinib producing a stronger suppressive effect than Cetuximab. These findings support MAPK inhibition as an effective strategy to inhibit EGFR-dependent invasive signaling.

Conclusion
It can be hypothesized that the regulation of LAMC2 concurs through MAPK signaling pathway.

Poster Group 4

P31

Synergistic Laminin-5/Integrin β4–EGFR Interaction Drives ERK-Dependent Invasion in Head and Neck Squamous Cell Carcinoma

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Objective

Head and neck squamous cell carcinoma (HNSCC) is a highly invasive cancer with poor clinical outcome. Resistance and aggressive tumor behavior highlight the necessity of elucidating the underlying molecular mechanisms. Integrin β4 (ITGB4) and Laminin-5 (LN5) are critical components of cell-extracellular matrix(ECM) communication. This work aims to understand their role in invasion and EGFR-related MAPK and focal adhesion (FAK) signaling.

Methods

Using FaDu wild-type (WT) and ITGB4 knockout (KO) cell lines, we performed invasion assays using 3D spheroids and Western blot under stimulation with EGF, LN5, and their combinations, along with MAPK and FAK signaling inhibition.

Results

EGF markedly induced invasion in WT which was effectively reduced by Cetuximab (EGFR inhibitor), Defactinib (FAK inhibitor), and Avutometinib (RAF/MEK inhibitor). LN5 enhanced EGF-induced invasion in both cell types, though KO cells show significant reduction of invasion. Defactinib alone produced limited effects on invasion, combined inhibition with Cetuximab or Avutometinib significantly suppressed invasion. Biochemically, EGF and LN5 induced pEGFR, pFAK and pERK activation, the latter is inhibited by Cetuximab and Avutometinib. LN5 did not activate classical EGFR tyrosine sites, but induced pERK. These data support a bifurcated model where EGF–EGFR–MAPK signaling acts as the primary driver, while LN5–ITGB4–FAK serves as a secondary pathway converging on ERK. The absence of ITGB4 impairs 3D invasive, indicating that ITGB4 provides structural anchorage in invasion. Inhibition of either FAK and MAPK signaling reduced invasion.

Conclusion

In summary, LN5 and ITGB4 interplay with EGFR-FAK/MAPK signaling, highlighting potential combination strategies targeting EGFR and integrin-associated signaling in HNSCC.

Fig. 1

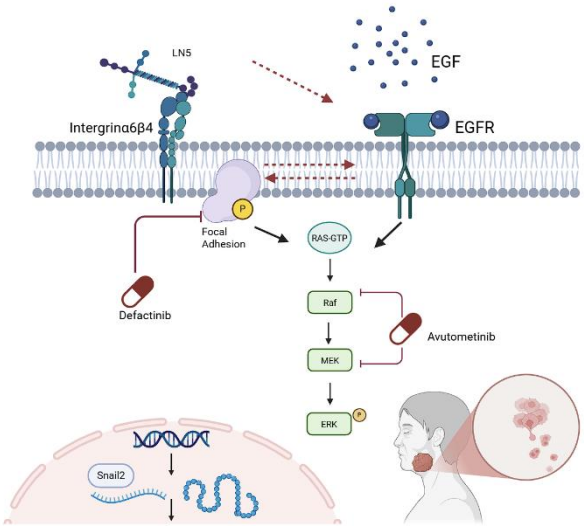
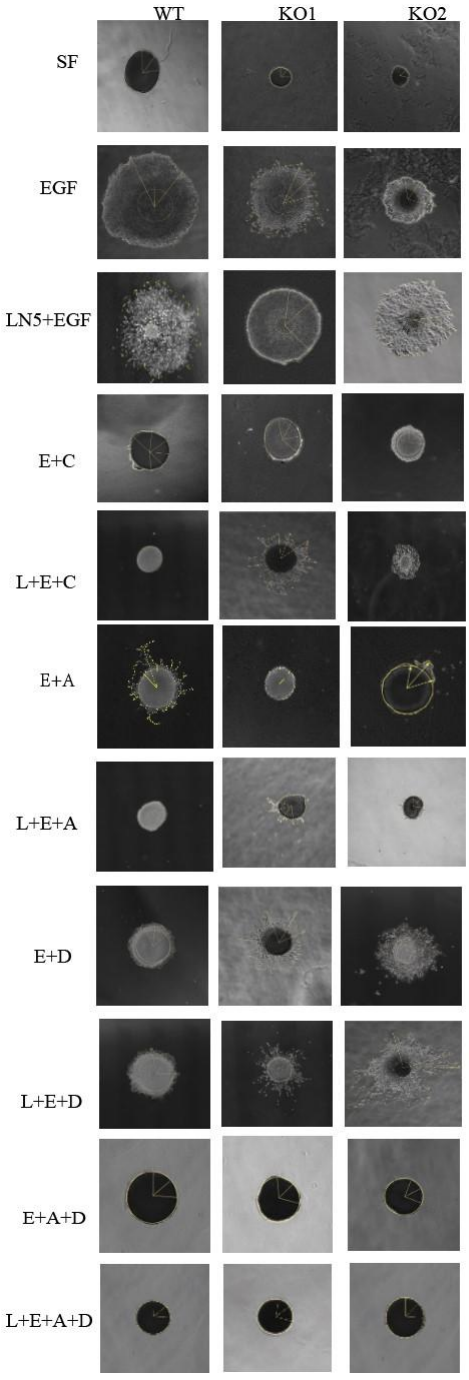


Fig. **2**



Poster Group 5

P32

HNSCC-Derived Extracellular Vesicles Impair Mitochondrial Respiration in Peripheral Immune Cells

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Introduction

Mitochondria are essential regulators of cellular energy metabolism, redox homeostasis, and immune cell activity. Tumor-derived extracellular vesicles (TEXs) contribute to tumor immune evasion in head and neck squamous cell carcinoma (HNSCC). TEXs carrying programmed death-ligand 1 (PD-L1) have emerged as potent mediators of immunosuppression. We investigated whether PD-L1–positive TEXs from HNSCC patient plasma impair mitochondrial function in peripheral blood mononuclear cells (PBMCs).

Methods

PBMCs from healthy donors were stimulated with IL-2 and cultured either alone or in the presence of PD-L1–positive TEXs, purified from patient plasma using size-exclusion chromatography (Exo-spin). Vesicle identity and PD-L1 expression were confirmed by nanoparticle tracking analysis and flow cytometry. Mitochondrial respiration was assessed and quantified by high-resolution respirometry (Oroboros) measuring oxygen consumption rates.

Result

PBMCs exposed to PD-L1–positive TEX showed a marked reduction in mitochondrial respiration compared with controls. This impairment indicates that these TEXs can directly compromise mitochondrial activity in immune cells, which may contribute to suppressed immune function within the tumor microenvironment.

Conclusion

PD-L1–positive TEX derived from HNSCC patient plasma suppress mitochondrial respiration in PBMCs, suggesting a mechanism of tumor-driven immune metabolic reprogramming. Further mechanistic analyses, including ongoing omics studies, are underway to extend these findings and highlight TEX-mediated immune metabolism as a potential therapeutic target.

Poster Group 5

P33

1,8-Cineole Targets the Adenosine A2a Receptor Pathway in Neutrophils to Modulate Tumor Immunosuppression in Head and Neck Cancer

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Despite advances in therapy, cancer remains a leading cause of mortality worldwide. Head and neck squamous cell carcinoma (HNSCC) is particularly challenging due to immune suppression, high recurrence, and treatment resistance. HNSCC remodels the tumor microenvironment (TME) into an immunosuppressive and thrombo-inflammatory niche that fosters tumor persistence and immune evasion. Overcoming this barrier requires approaches that not only target tumor cells but also reprogram immune and thrombo-inflammatory circuits.

1,8-Cineole (eucalyptol), a phytochemical monoterpene, has shown dual anti-tumor and anti-inflammatory effects. Preclinical studies have demonstrated that cineole inhibits tumor growth, induces apoptosis, and suppresses inflammatory mediators; however, its immune-related mechanisms in HNSCC remain poorly understood.

We demonstrate that cineole modulates the adenosine A2A receptor (ADORA2A) in neutrophils, dampening oxidative burst and degranulation, thereby attenuating neutrophil-driven immunosuppression in the TME. Supporting this mechanism, proteomic analyses in platelets revealed that cineole activates the ADORA2A–cAMP–PKA–VASP pathway to prevent platelet activation and aggregation, establishing ADORA2A as its direct target.

By targeting ADORA2A in both neutrophils and platelets, cineole addresses two critical barriers in HNSCC: immune evasion and pro-thromboinflammatory signaling. Targeting this pathway may enhance therapeutic responsiveness, reduce recurrence, and attenuate immunothrombosis, supporting the development of 1,8-Cineole as a novel immunoregulatory strategy in HNSCC.

Poster Group 5

P34
T Cell Landscape in Head and Neck Cancer

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Objective
Head and neck squamous cell carcinomas (HNSCCs) exhibit a variety of immune escape mechanisms, including tolerance to cytotoxic T cells, the expression of inhibitory immune checkpoint receptors and the generation of regulatory T cells(1). Our aim is to elucidate the role of T cells in HNSCC progression and how they interact with tumor cells.

Methods
Single-cell RNA sequencing of HNSCC specimens from distinct anatomical localizations was performed. T cells were classified into subpopulations according to their activity status and function. Interactions with tumor cells were predicted by ligand-receptor interactions and the T cell role in the tumors was further analyzed by pathway enrichment analysis. A spatial proteomics approach was used to evaluate the localization of T cells within the tumor.

Results
The T cell landscape was dominated by T helper cells and regulatory T cells. Cytotoxic T cells were predominantly in an exhausted state, as determined by their decreased cytotoxicity and elevated expression of immune checkpoints. CD8+ T cells, which are associated with chronic inflammation were identified. Furthermore, we identified T cell populations which are associated with the formation of tertiary lymphoid structures (TLS) within the tumor. TLS were visible in the tumor tissue sections.

Conclusion
Our present findings suggest that the landscape of T cells in HNSCC is characterized by exhaustion and disfunction, which is largely caused by the tumor cells. Large numbers of regulatory T cells were identified. T cells appear to promote the formation of TLS within the tumor, as evidenced by the presence of TLS in the tissue sections.

(1) Damasio, MPS, Nascimento, CS, Andrade, LM, de Oliveira, VL, Calzavara-Silva, CE: The role of T-cells in head and neck squamous cell carcinoma: From immunity to immunotherapy. Frontiers in Oncology, Volume 12 - 2022, 2022. https://doi.org/10.3389/fonc.2022.1021609

Poster Group 5

P35

Effect of High Hydrostatic Pressure-treated HNSCC Cells on CAF-like Differentiation of Adipose-derived Stromal Cells

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Cancer-associated fibroblasts (CAFs) are key mediators of tumor–stroma interactions in head and neck squamous cell carcinoma (HNSCC). They can arise from mesenchymal stromal cells (MSCs) under the influence of tumor-derived soluble factors. High hydrostatic pressure (HHP) treatment enables complete devitalization of tumor-infiltrated tissue while preserving extracellular matrix integrity, making it a promising approach for preparing autologous grafts after tumor resection. To assess the oncological safety of this method, this study examined whether soluble mediators from HHP-treated HNSCC cells can induce CAF-like activation of human adipose-derived stromal cells (hASCs).

HNSCC cell lines (HNSCC16, UTSCC14) were treated at 300 MPa for 10 min to achieve devitalization. Conditioned media (CM) from untreated and HHP-treated cells, as well as TGF-β1 stimulation (10 ng/mL, positive control), were applied to hASCs for 96 h. CAF marker expression (αSMA, FAP, TNC) was analyzed by qRT-PCR, immunofluorescence, and Western blot. Morphological changes were assessed, and cytokine profiling of CM and hASC supernatants was performed using dot blot and multiplex assays.

Untreated tumor CM induced moderate upregulation of CAF markers, whereas CM from HHP-treated tumor cells did not significantly alter gene or protein expression compared with controls. Although hASCs exposed to HHP-treated CM showed partially altered morphology, no induction of αSMA, FAP, or TNC expression was detected. TGF-β1 stimulation led to strong upregulation of all CAF markers, confirming the general differentiation potential of hASCs.

HHP treatment at 300 MPa markedly reduces the ability of HNSCC-derived soluble factors to activate stromal cells. These findings suggest that HHP-devitalized tumor-conditioned media do not promote CAF-like differentiation of hASCs, supporting their suitability for reconstructive applications after tumor resection. Further in vivo studies are needed to confirm these results within the complex tumor microenvironment.

Poster Group 5

P36

Head and Neck Squamous Cell Carcinoma-derived Small Extracellular Vesicles Mediate Ca^{2+}-dependent Platelet Activation and Aggregation through Tissue Factor

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Background
Head and neck squamous cell carcinoma (HNSCC) is an aggressive disease with poor prognosis, driven by recurrence and metastasis. Tumor heterogeneity and an immunosuppressive microenvironment are key contributors. Tumor-derived extracellular vesicles (EVs) modulate the microenvironment by transferring bioactive components to platelets, initiating platelet activation and thromboinflammation that support tumor progression.

Methods
EVs were isolated from SAS and UD-SCC-5 HNSCC cell lines by size exclusion chromatography and characterized by electron microscopy, nanoparticle tracking analysis, flow cytometry, and Western blotting. Platelet activation and aggregation were assessed by aggregometry, flow cytometry, and ELISA. Mechanisms were investigated through proteomic analysis, thrombin generation assays, and inhibitor studies.

Results
HNSCC-derived EVs activated and aggregated platelets in a **calcium-dependent manner**. Proteomic analysis revealed **tissue factor** within EVs, triggering coagulation and delayed but robust platelet aggregation. Inhibiting thrombin activity or its receptors significantly reduced aggregation, demonstrating the pivotal role of tissue factor–dependent thrombin generation in platelet activation. EVs thus establish a prothrombotic milieu through tissue factor–driven mechanisms.

Conclusion
Tumor-derived EVs critically modulate platelet function and promote thrombogenesis in HNSCC. Targeting EV-mediated platelet activation may offer novel therapeutic strategies to mitigate thrombotic complications. In vivo studies could clarify their clinical relevance.

These data have already been published: Weiser, T., *et al.* - Head and neck squamous cell carcinoma-derived extracellular vesicles mediate Ca^{2+}-dependent platelet activation and aggregation through tissue factor. *Cell Commun Signal* **23**, 210 (2025)

Poster Group 5

P37
Extracellular Vesicles Modulate Immune Cell Function and Metabolism

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Introduction
Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer globally. Tumor-derived extracellular vesicles (TDEVs) contribute to the immunosuppressive microenvironment by promoting intercellular communication, platelet activation, and tumor progression. This study aims to characterize TDEVs from HNSCC cell lines (UD5, SAS) and patient-derived EVs, focusing on their molecular profiles and interactions with immune cells.

Methods
Peripheral blood mononuclear cells (PBMCs) from healthy donors were stimulated with low-dose IL-2 for four days prior to immunophenotyping. TDEVs were purified by size-exclusion chromatography. Surface marker expression, vesicle size, and morphology were assessed by flow cytometry, nanoparticle tracking analysis, and electron microscopy. Hsp70 binding to the cmHsp70.1 antibody was measured by microscale thermophoresis. For functional analysis, TDEVs were co-incubated with PBMCs, and mitochondrial oxygen consumption (O_2 flux) was measured using high-resolution respirometry.

Results
TDEVs showed consistent size distribution, expressed exosomal markers (CD9, CD63, CD81), and bound strongly to cmHsp70.1. Electron microscopy validated morphology. Functionally, co-incubation with TDEVs induced immunosuppression, including reduced expression of activating NK cell receptors such as NKG2D. PBMCs exposed to cell line-derived EVs exhibited reduced mitochondrial respiration, while patient-derived EVs elicited a more variable, milder response, indicating donor-dependent functional heterogeneity.

Discussion
TDEVs suppress immune function by downregulating activatory NK receptors and impairing PBMC mitochondrial respiration. The extent of these effects differed by EV origin, suggesting that surface markers and molecular cargo shape their immunomodulatory potential. These findings highlight the importance of EV-mediated immune regulation in HNSCC and support targeting EV-associated pathways to counteract immune evasion.

Poster Group 5

P38
HNSCC-Derived PD-L1 Positive Exosomes Reprogram Platelet Mitochondrial Respiration

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Head and neck squamous cell carcinoma (HNSCC) is a highly aggressive malignancy with poor survival outcomes, largely due to its persistent ability to suppress immune activation and drive tumor progression. Programmed death ligand 1 (PD-L1) is frequently overexpressed in HNSCC and transferred to other cells within the tumor microenvironment (TME). Platelets are increasingly recognized as key regulators of the TME, where they promote tumorigenesis and inhibit antitumor immunity. Beyond local effects, platelets can be "educated" by tumor cells through exosomes, acquiring tumor-derived signatures that may disseminate systemically. However, the impact of PD-L1–positive tumor-derived exosomes on platelet function remains largely unexplored.

To investigate this, we employed FaDu PD-L1-/+ as models and exposed their isolated exosomes to isolated platelets from healthy donors. Exosomes were purified using the Exo-spin™ size exclusion chromatography system and characterized in accordance with MISEV criteria by nanoparticle tracking analysis, transmission electron microscopy, flow cytometry, and Western blot. PD-L1 expression was quantified on tumor cell membranes as well as exosomes.

Functionally, we assessed the effects of PD-L1–positive exosomes on platelet metabolism using high-resolution respirometry (Oroboros O2k). Our data demonstrated that PD-L1–positive tumor-derived exosomes suppress platelet mitochondrial respiration more strongly than PD-L1-negative controls. We further characterized mitochondrial ultrastructure via transmission electron microscopy and applied multiomic analyses to delineate deregulated platelet metabolic pathways driven by PD-L1 signaling. To confirm PD-L1"s role, we repeated the experiments on RaJi PD-L1-/+ cells and obtained similar results.

In conclusion, PD-L1–positive exosomes from HNSCC suppress platelet mitochondrial respiration, indicating that platelet metabolism represents a novel axis of tumor–host interaction with therapeutic relevance.

Poster Group 5

P39

Blood-Based Inflammatory Markers are Associated with Treatment Outcomes in Head and Neck Squamous Cell Carcinoma Receiving Anti-PD-1 Therapy: CRP as a Superior Predictive Marker

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Background
Systemic inflammation is gaining attention as a potential predictive biomarker in immune checkpoint inhibition (ICI) for head and neck squamous cell carcinoma (HNSCC). Several blood-based inflammatory markers estimate systemic inflammatory burden, but their value in predicting ICI outcomes remains unclear.

Methods
We evaluated the predictive role of C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune inflammation index (SII) in HNSCC patients receiving anti-PD-1 monotherapy. Seventy-nine patients were included in this retrospective study. Optimal cutoffs were defined by receiver operating characteristic analysis to stratify patients into high vs. low inflammation groups. Chi-square tests assessed response differences. Progression-free survival (PFS) and overall survival (OS) were analyzed by Kaplan-Meier and log-rank tests, and by uni- and multivariable Cox regression.

Results
Elevated CRP correlated with a reduced disease control rate. In univariable analysis, high-inflammation groups had significantly worse OS and PFS across all indices. In multivariable analysis, CRP and combined positive score remained independent predictors of OS and PFS.

Conclusion
High systemic inflammation was associated with poorer outcomes during anti-PD-1 therapy in HNSCC. CRP emerged as the most robust independent biomarker and a potential predictor of OS and PFS in these patients.

Poster Group 6

P40

Development and Evaluation of FPR1 Targeted Bacterial Signal Peptide Radiotracers for Radiopharmaceutical Use

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Bacterial signal peptides constitute a diverse class of high-affinity ligands for formyl peptide receptors (FPRs), which are abundantly expressed on glioblastomas as well as other malignancies such as colon and ovarian cancers. FPR1 exhibits up to 30-fold increased surface expression in cancer cells, while its ligands have affinities in the low picomolar range, making FPR1 an attractive target for molecular imaging.In this study, we investigated bacterial signal peptides as a relatively unexplored class of high-affinity FPR agonists for radiopharmaceutical applications. To identify modification sites compatible with receptor binding, we first synthesized fluorescence-labeled derivatives and tested them using confocal microscopy. From this, we developed a selective fluorescence probe with a >1000-fold preference for FPR1, rapid binding at low nanomolar concentrations, stable receptor-ligand complex formation for up to 72 hours, and effective penetration into U87 glioma spheroids. Based on these findings, we developed a radiotracer by conjugating a formylated signal peptide with Ahx-DOTA to chelate it with gallium (Ga) or lutetium (Lu).The resulting metal chelate-peptide conjugates retained their affinity and were efficiently taken up by both FPR1-transfected HEK293T cells and naturally FPR1-expressing U87 cells. A pilot study of planar scintigraphy in healthy mice showed minimal nonspecific uptake and no significant retention in major organs. These results indicate that bacterial signal peptides are promising candidate for the development of targeted FPR1 radiopharmaceuticals with great potential for applications in positron emission tomography (PET) and single photon emission computed tomography (SPECT).

Poster Group 6

P41
Microbial-Neutrophil Interplay in the Malignant Oral Mucosa

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The oral microbiota is a dynamic community, and dysbiosis has been linked to various diseases including cancer. Additionally, mucosal surfaces and oral tumors are highly colonized by bacteria, suggesting that the oral microbiome might contribute to head and neck cancer (HNC) development by modulating interactions among stroma, tumor, and immune cells. Therefore, characterization of the microbiome in malignant oropharyngeal tissues could support advances in cancer treatment.

Here, using 16S RNA sequencing and immunofluorescence microscopy, we characterized the oral microbiota and immune cells in human oral cavity cancer. We found that the microbiota in malignant mucosa showed increased diversity and a different composition compared to normal mucosa of HNC patients, with Fusobacterium *nucleatum* (Fn) as the most abundant bacterium. Moreover, tissue analysis showed bacteria accumulated more in tumor parenchyma than stroma. Intra-tumoral bacteria formed clusters with neutrophils and tumor cells undergoing partial epithelial-mesenchymal transition (pEMT). Neutrophils in these clusters produced neutrophil elastase (NE), matrix metalloproteinase-9 (MMP-9), and myeloperoxidase (MPO).

Then, using in vitro systems that model the interaction between tumor and immune cells during bacterial stimulation, we tested bacterial effects. Staphylococcus *aureus*-primed tumor cells induced rapid formation of neutrophil extracellular traps (NETs)s and secretion of NE and MMP-9 in neutrophils, triggering an aggressive tumor phenotype with increased invasion, migration, and metastasis. Meanwhile Fn-primed tumor cells increased neutrophil recruitment, prolonged their survival, induced MPO secretion, and reduced neutrophil phagocytic capacity.

In conclusion, these results suggest that intratumoral bacterial stimulation may generate a pro-metastatic cross-talk between tumor and immune cells and represents a previously unknown driver of HNC progression.

Poster Group 6

P42

Mucosal Vaccine Synergizes with THBS1 Inhibition for Enhanced Anti-Tumor Efficacy in HPV+ OPSCC

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This study investigates a novel mucosal vaccine for HPV-associated oropharyngeal squamous cell carcinoma (HPV+ OPSCC), utilizing an adenoviral vector encoding multimeric HPV antigens delivered via intranasal administration. Through single-cell sequencing and in vivo/in vitro experiments, we demonstrate that the vaccine dually activates systemic and mucosal tissue-resident memory T cells (TRM), effectively suppressing tumor progression. However, the vaccine concurrently induces tumor cells to secrete CCL2, recruiting Thbs1+ monocytes to the tumor microenvironment via the CCL2-CCR2 axis. These monocytes exhibit dual roles: they exacerbate TRM exhaustion, impairing cytotoxic function, while simultaneously promoting tumor stemness and proliferation. To counterbalance this paradoxical mechanism, we combined the mucosal vaccine with a THBS1 inhibitor, achieving synergistic enhancement of anti-tumor efficacy. Our findings highlight the vaccine"s potential to mobilize both adaptive and innate immunity while underscoring the necessity of targeting monocyte-driven immunosuppression for optimal therapeutic outcomes. This combination strategy provides a promising translational approach for HPV+ OPSCC treatment, addressing both tumor clearance and microenvironmental resistance.

Poster Group 6

P43

Analysis of Oxidative Stress and Metabolic Reprogramming in HPV⁺ Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinoma (HNSCC) associated with human papillomavirus (HPV) infection display altered energy metabolic pathways due to numerous oncogenic events activated by viral oncoproteins (E6 and E7) and their splice variants. A subgroup of HNSCC presents with high expression levels of E6*I, the major splice variant of E6, and correlates with oxidative and metabolic stress pathway signatures indicative for unfavorable prognosis. Here, we aimed to understand the effects of E6*I overexpression on oxidative stress (OS) defense and metabolic pathways.

HEK 293 cells overexpressing HPV16-E6*I-GFP and -E6-GFP were established and subjected to in vitro characterization by mimicking the atmospheric O_2 conditions of the healthy and tumor tissue (5% O_2 normoxia and 2% O_2 hypoxia). The effects of E6 and E6*I on OS defense pathway components and metabolic reprogramming were studied by monitoring the expression and subcellular localization of viral proteins, immunofluorescence, live-cell imaging, ddPCR and protein expression analysis of key energy metabolic enzymes as well as cell metabolic assays.

Studying cells treated at different O_2 concentrations, ROS and metabolic markers showed highly divergent expression in control and E6 compared to E6*I overexpressing cells. This highlights a distinct role of E6*I and the dependence of viral oncogene expression on oxygenation and ROS production in HPV⁺ tissue. Particularly, under hypoxic conditions, overexpression of E6*I was associated with increased proliferation, increased expression of OS defense components, and altered glycolysis and OXPHOS activity.

In summary, in vitro analysis of E6*I overexpression revealed signatures of OS defense and metabolic reprogramming, which were also observed in a subset of HNSCC patients presenting with E6*I overexpression, viral host genome integration, and unfavorable prognosis. Testing for E6*I expression in patient samples could be of interest in the future to determine prognostically and therapeutically relevant subgroups.

Poster Group 6

P44
Reconstructive Surgery of the Midfacial Zone in Patients with Maxillary Cancer

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Keywords
oral cancer, maxillary cancer, middle face zone reconstruction

Over the past decade, Ukraine has experienced a steady annual increase in the incidence of malignant tumors of the oral cavity. In 2018, according to data from the Health Statistics Center of the Ministry of Health of Ukraine, 2,127 individuals were diagnosed with oral tumors. The National Cancer Registry of Ukraine reported that between 2020 and 2024, a total of 5,300 patients with oral cancer were newly recorded. In 2024, most cases (39.7%) were diagnosed at stage IV, and 31.4% of the patients did not survive the year by operational data.

Objective
The use of the author"s original plastic surgery technique for defect reconstruction following maxillary resection resulted in significant physiological and aesthetic improvements in patients" quality of life.

Materials and Methods
This study encompassed 99 patients aged 31–84 years diagnosed with maxillary cancer, who were treated at the ENT Oncopathology Department of the O.S. Kolomiychenko Institute of Otolaryngology, NAMS of Ukraine, during the period from 2014 to 2019.

Results
A group of patients underwent maxillary resection with simultaneous plastic reconstruction of the orbital floor defect using a musculoskeletal graft harvested from the coronoid process and anterior edge of the mandibular ramus, according to our original technique. This approach offers several advantages.

First, it allows for immediate reconstruction of the orbital floor, preventing downward displacement of the eyeball and the development of diplopia – complications that are difficult to correct at a later stage. Second, the transplantation of a healthy block of bone and muscle into the area subsequently exposed to chemotherapy and radiation therapy significantly improves postoperative outcomes.

Reconstruction of the hard palate and the alveolar process of the maxilla was performed during the second stage of treatment, 6 to 12 months after the initial radical surgery.

Conclusion
The author"s technique enables patients to maintain their natural facial contour and prevents downward displacement of the eyeball. This method is indicated in cases where complete tumor removal can be guaranteed. Early diagnosis plays a crucial role in achieving favorable treatment outcomes for this category of patients.

Poster Group 6

P45

Head-to-Head Comparison of 18F-PSMA and 18F-FDG PET/CT in Locoregionally Advanced Head and Neck Squamous Cell Carcinoma

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Objective

Positron emission tomography/computed tomography (PET/CT) with 18-fluorine-fluorodeoxyglucose (18F-FDG), a marker of glycolytic activity, has been used for staging of head and neck squamous cell carcinoma (HNSCC), but prostate-specific membrane antigen (PSMA) PET/CT, targeting tumor neovasculature, may offer complementary insights. Thus, this study aimed to compare FDG PET/CT to 18F-PSMA-1007 PET/CT images in patients with HNSCC.

Methods

Fourteen patients with advanced HNSCC underwent scans using a dedicated PET/CT scanner (Biograph mCT40, Siemens Healthcare, USA). Both scans were performed within a 24-hour interval to ensure temporal proximity of findings. Detection rates and SUVmax were analyzed.

Results

FDG PET/CT detected primary/metastatic lesions in all patients. Ten out of 13 (76.9%) patients with unresected primary lesions exhibited 18F-PSMA-1007 uptake. Among 13 patients with FDG-avid metastatic lesions, 6 (46.2%) showed corresponding uptake on PSMA imaging. The patients with the largest tumors presented intralesional patterns differ markedly. For primary tumors, mean SUVmax values obtained at 1 hour and at 2 hours with FDG PET/CT were higher than those observed by PSMA PET/CT (25.6 ± 16.4 vs. 4.6 ± 1.4, 30.0±16.9 vs. 5.4±1.5, respectively, $p < 0.001$).

Conclusions

Our data indicate: 1) HNSCC lesions were better detected by 18F-FDG PET/CT than by 18F-PSMA-1007 PET/CT, 2) The uptake of both tracers in most tumors suggests that glycolytic activity and neoangiogenesis may coexist, and 3) The unique angiogenic information provided by 18F-PSMA-1007 could be instrumental in tailoring patients with HNSCC for targeted therapies. Acknowledgments: The study was supported by São Paulo Research Foundation (FAPESP, Cancer Theranostics Innovation Center, #2021/10265-8); and National Council for Scientific and Technological Development, CNPq, Research Productivity, Grant # 312212/2025-9 (Ramos CD), Grant # 302922/2025-3 (Lima CSP).

Poster Group 6

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Targeted Molecular Imaging of Head and Neck Cancer Using a Ga-68-labeled Anti-Integrin Peptide

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Objective
The present study evaluated the effects of the anti-integrin peptide, developed by FAPESP-founded CancerThera, on cell proliferation, cell cycle, apoptosis, binding and internalization as a first step toward targeted head and neck cancer (HNC) theranostics.

Methods
HNC cells (FaDu, SCC-9, and SCC-25) were used. Anti-proliferative activity of the anti-integrin peptide (0.01 nM–100 µM) was tested by comparing cell density at baseline and 48h using sulforhodamine B staining (540 nm). [68Ga]Ga-anti-integrin was labeled in sodium acetate buffer (pH 5.5, 95°C, 10 min) and purified via Sep-Pak C18. Radiochemical yield was assessed by iTLC-SG using methanol/ammonium acetate as mobile phase. Cell cycle and apoptosis were analyzed by flow cytometry. Binding and internalization assays were performed at 30 and 60 min of incubation, with radioactivity quantified using a gamma counter.

Results
At the tested concentration range, the anti-integrin peptide did not affect HNC cells proliferation, cell cycle or apoptosis. FaDu, SCC-9, and SCC-25 at 30 min revealed binding rates of 9.0% ± 0.8%, 8.9% ± 2.7%, 10.4% ± 1.7%, and internalization rates of 8.3% ± 1.5%, 9.9% ± 1.6%, 12.2% ± 1.4%, respectively. At 60 min, binding percentages were 6.8% ± 0.5%, 10.2% ± 2.7%, 8.5% ± 0.7%, while internalization values were 9.2% ± 0.7%, 10.0% ± 2.0%; 9.9% ± 1.4%, respectively.

Conclusion
Overexpressed in HNC, integrins are transmembrane proteins that play essential roles in HNC and may therefore represent a novel approach for patient monitoring. The anti-integrin peptide showed no effect on cell viability, and [68Ga]Ga-anti-integrin peptide exhibited an important affinity for HNC cells, suggesting potential to improve the assessment of diagnosis, therapy response, and tumor progression. Nevertheless, in vivo studies are essential to validate its theranostic potential.

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Poster Group 6

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Implant-Retained Silicone Facial Prosthesis, a Small Experience from Digital Design to Material Safety

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Background
Reconstructive challenges become more complex after oncologic resection of extensive orbital and midfacial defects. When contour and esthetics cannot be restored with traditional flaps, implant-retained silicone prostheses offer a minimally invasive approach to restoring facial form and function. This study presents a seven-year evolution (2017–2025) in surgical design, digital fabrication, and follow-up maintenance.

Methods
Extraoral implants were placed in a consecutive series of post-resection patients for magnet-retained silicone prostheses. Key developments included: magnetic retention with dental implants, enhanced silicone–magnet adhesion using a plastic clay interface, 3D-guided fabrication with reproducible molds, prototype-based personalization to minimize fittings, and SEM–EDS analysis of a failed implant revealing elemental contamination. Patients were reviewed every 3–6 months for oncologic surveillance and peri-implant health, including hygiene assessment and scheduled silicone replacement.

Results
All prostheses achieved stable osseointegration (ISQ > 65) with excellent long-term retention. Reproducible mold protocols reduced fabrication time by 40%, and patients reported high esthetic and functional satisfaction. SEM–EDS analysis identified metal contamination associated with peri-implant irritation, prompting new preventive hygiene and monitoring protocols. No recurrent peri-implantitis or cancer was observed during structured follow-up.

Conclusion
Over seven years, surgical innovation integrating digital fabrication, personalized prosthesis design, and continuous follow-up has enhanced rehabilitation outcomes in cancer-related defects. Implant-supported silicone prostheses represent a precise and sustainable reconstructive strategy emphasizing patient safety, function, and long-term well-being.

Keywords
Silicone facial prosthesis, implant-retained orbital prosthesis, 3D-guided fabrication, SEM–EDS, long-term follow-up