ABSTRACTS

2ND INTERNATIONAL MEETING ON PRECISION ONCOLOGY AND PERSONALIZED MEDICINE FOR HEAD AND NECK CANCER

17–18 January 2025 Heidelberg

www.headandneck-cancer.de

2nd International Meeting on Precision Oncology and Personalized Medicine for Head and Neck Cancer

with scientific support the

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

Conference Chairs

Prof. Dr. Jochen Hess
Department of Otorhinolaryngology,
Head and Neck Surgery
University Hospital Heidelberg

Dr. rer. nat. Ina Kurth
Head Service Unit for Radiopharmacy and
Morszeck Preclinical Trial Unit (PCTU)
Laboratory Head Division RadioOncology/
RadioBiology

Working Group Oncology oft he German Society of Oto-Rhino-Laryngology, Head and Neck Surgery

Jens Peter Klußmann
University Hospital Cologne
Department of Oto-Rhino-Laryngology,
Head and Neck Surgery

Stephan Lang
University Hospital Essen
Department of Oto-Rhino-Laryngology,
Head and Neck Surgery

Scientific Board

Editor: Prof. Dr. Jochen Hess
ISBN: 978-3-948023-50-8

Table of content

Session 1: Epidemiology, Prevention and Diagnose 5
L1 Comparative analysis of saliva- and plasma-derived small extracellular vesicles from head and neck cancerpatients from a liquid biomarker perspective 5
L2 Proof of concept study for HPV16 E6 serology based screening and early detection of HPV-driven oropharyngeal cancer 6
L3 Identification of potential mRNA-biomarker for molecular non-invasive OSCC diagnostic and therapy control 7

Session 2: Immunotherapy, Cellular Therapy and Vaccination 8
L4 Marginal Zone B cells act as professional antigen-presenting cells in head and neck squamous cell carcinoma 8
L5 Glutamine synthetase expression enhances T cell effector functions in a glutamine-restricted environment 9
L6 The effect of HNSCC-derived exosomes on the immunological function of neutrophil granulocytes 10

Session 3: Interdisciplinary Tumor Board 12
L7 Molecular Insights into tumor budding in HNSCC: A multi-omics exploration 12
L8 Functional kinome profiling for personalized treatment of head and neck cancer 13
L9 Tumor tissue slices – A powerful translational tool for precising head and neck cancer radiotherapy 14
L10 De-escalation of adjuvant radio(chemo)therapy for patients with HPV-positive oropharyngeal squamous cell carcinoma (DELPHI study) 15

Session 4: Radiooncology and Imaging 16
L11 Predicting immunometabolic status in head and neck squamous cell carcinomas using multiparametric PET/MRI: Study plan and preliminary validation of biomarkers 16
L12 Tissue microarray analyses of the essential DNA repair factors ATM, DNA-PKcs and KU80 in head and neck squamous cell carcinoma 17
L13 CD44 transcript variants as determinants of HNSCC radioresistance: Metabolic implications and therapeutic vulnerabilities 18

Session 5: Molecular Landscape, Multiomics and Artificial Intelligence 19
L14 Personalized cell-free circulating tumour DNA analysis for patients with HNSCC 19
L15 Single-cell spatial analysis of the salivary gland carcinoma microenvironment 20
L16 Molecular landscape of oral cancer in young adults 21

Session 6: Mechanisms of Treatment Failure and New Drug Targets 22
L17 EGFR-mediated local invasiveness and response to cetuximab in head and neck cancer 22
L18 Mechanistic study of CCDC86 promoting the development of oral squamous cell carcinoma by regulating ribosome biogenesis 23
L19 Combined treatment of HPV-positive HNSCC cells with xevinapant, tuvusertib and ionizing radiation 24

Session 7: New Technologies and Preclinical Models 25
L20 Patients-derived (PD) cultures as preclinical models for tumor microenvironment 25
L21 DARPin induced reactivation of p53 and its therapeutic potential for treatment of HPV induced tumors 26
L22 Label-free histopathology using SRS microscopy and AI for head and neck cancer evaluation 27

Poster Session 29
P1 Mitochondrial gene signatures in the sex-specific pathogenesis and therapy of head and neck tumors....29
P2 Tracking tumor heterogeneity in head and neck squamous cell carcinoma (HNSCC).....30
P3 The mitochondrial plasticity in the pathogenesis and therapy of HPV16-negative head and neck tumors 31
P4 Silencing PTBP1 reduces HNSCC cell line proliferation.....32
P5 Modulation of tumor metabolism to improve antitumor immune response in HNSCC.....33
P6 Platelet-mediated induction of epithelial-mesenchymal transition in head and neck squamous cell carcinoma.....34
P7 Innovative gold-based nanocarrier strategy to enhance therapy in head and neck squamous cell carcinoma.....35
P8 Anti-proliferative activity of a palladium(II) complex over squamous cell carcinoma of tongue.....36
P9 In vitro conditioning of NK cells through exposure to HNSCC-derived exosomes.....37
P10 Intratumoral T-cell abundance and antigen specific immune responses in HPV positive and negative head and neck cancer.....38
P11 Two distinctive molecular subgroups of patients with specific expression phenotypes determine the majority of recurrences in advanced laryngeal carcinoma.....39
P12 Neural progenitors driving neurogenesis in head and neck cancer and its role in tumor development 42
P13 miR-4421 as a possible modulator of MAPK/Akt pathway through ERP29 in pharyngeal cancer 43
P14 Effect of ERP29 silencing on PI3K/AKT pathway gene expression in cisplatin-sensitive and resistant pharyngeal cancer cells 44
P15 Adipocytes enhance tongue cancer progression: Possible involvement of adipokine IL-6 and extracellular vesicles 45
P16 Self-supervised analyses of head and neck cancer histologies reveal novel malignant growth pattern.....46
P17 Reevaluation of the immunotherapy Bayesian network model for head and neck cancer 47
P18 Spatial distribution of leukocyte subsets affects development of head and neck squamous cell carcinoma.....48
P19 Mapping molecular subtypes in HNSCC: From bulk RNA to spatial data 49
P20 Impact of the sex chromosome dosage on the tumor microenvironment in head and neck squamous cell carcinoma.....50
P21 Impact of combined SNVs on renal function in HNSCC patients undergoing Cisplatin-based treatment....51
P22 Presence of HPV peptides in small extracellular vesicles released by tumor cancer cells in vitro and in vivo 52
P23 Trends in epidemiology and prevention strategies for head and neck cancer: Insights from a multinational cohort study 54
P24 Effects of financial strain on quality of life in German head and neck cancer survivors 55
P26 Detection of methylated tumor markers in head and neck cancer and tumor environment.....56
P27 Longterm outcome of patients with early stage glottic or supraglottic cancer – As good as it seems? 57
P28 Platin-based chemoradiotherapy as definitive treatment in advanced squamous cell carcinoma of head and neck 58
P29 Docetaxel and cisplatin induction chemotherapy with or without fluorouracil in locoregionally advanced head and neck squamous cell carcinoma: A real-world data study.....59
P30 Overview of murine head and neck squamous cell carcinoma models for lymphatic and haematogeneous metastasis as well as for primary tumour induction in immunocompetent mice 60
P31 Investigation of tumor-associated macrophages in different anatomical sites of head and neck cancer ...61
P32 Effect of the isolation method on the properties of head and neck squamous cell carcinoma (HNSCC) cells in preclinical models 62
P33 Patient derived tumour fragments as pre-clinical ex vivo models for HNSCC 63
P34 Development of an orthotopic *in vivo* model for the combination of therapeutic vaccination with low-dose irradiation in HPV-driven oropharyngeal cancers.....64
P35 Combining head and neck cancer tissue slice cultures with sequential immunofluorescence for functional drug testing 65
P36 Establishment of differentiation therapies targeting squamous cell carcinomas of the head and neck and other locations 66
P37 Propagator methods for survival analysis 67
P38 Evaluating the CAM assay as a preclinical model for head and neck tumors: Engraftment efficiency, xenografts and serial transplantation.....68

P39 Identification of distinct subpopulations of cancer associated fibroblasts in oral squamous cell carcinoma by imaging mass cytometry....69
P40 Characterization of irradiated mucosa using confocal laser endomicroscopy in the upper aerodigestive tract....70
P41 Double Trouble: Identifying optimal combinations for the IAP-inhibitor Debio 1143 for the radiosensitization of HNSCC cell lines and tissue slices....71
P42 Diagnosis of lymph node metastases in head and neck cancers using Hsp70-specific fluorescence imaging....72
P43 Implications of the partial volume effect correction on the spatial quantification of hypoxia and clonogenic cell density based on [18F]FMISO and [18F]FDG PET/CT data....74
P44 Molecular imaging-guided radiotherapy of the head and neck has the potential to enhance treatment tolerability....75
P45 Evaluation of auto-segmentation solutions for lymph node level delineation in head and neck cancer radiation therapy....77

Epidemiology, Prevention and Diagnose

L1

Comparative analysis of saliva- and plasma-derived small extracellular vesicles from head and neck cancer patients from a liquid biomarker perspective

S. Ludwig[1], J. Schütz[1], F. Jungbauer[1], A. Lammert[1], M. N. Theodoraki[2], E. Seiz[1], C. Scherl[1], N. Rotter[1], L. Tengler[1]

[1]Medical Faculty Mannheim of Heidelberg University, Department of Otorhinolaryngology, Head and Neck Surgery, Mannheim, Germany
[2]Klinikum rechts der Isar, Technical University Munich, Department of Otorhinolaryngology, Head and Neck Surgery, Munich, Germany

Question: Saliva is a non-invasive source of small extracellular vesicles (sEVs), that are highly exposed to Head and Neck cancers (HNC) compared to plasma-derived sEVs (PEVs). A comprehensive characterization of these vesicles could provide a valuable additional liquid biomarker source.

Methods: sEVs from saliva (SEVs) were isolated via ultracentrifugation and from plasma (PEVs) by size-exclusion chromatography from 19 HNC patients and 8 healthy donors as controls (HDs). sEVs were characterized according to the consensus criteria (MISEV 2023) including transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). EV-markers and immunoregulatory protein patterns were analyzed by western blots and flow cytometry. Functional assays were used to determine the immunomodulatory effect of SEVs on CD8+ T cells and NK cells and compared with PEV-mediated effects.

Results: In TEM, SEVs and PEVs showed the typical morphology of sEVs. NTA detected higher amounts of smaller vesicles in plasma than saliva (median Ø 71 vs. 111nm). Both, SEVs and PEVs from HNC patients carry immunosuppressive proteins (PD-L1, TRAIL, CTLA-4) with particularly elevated PD-L1+ and FasL+ levels on HPV-negative/advanced-stage HNC SEVs ($p<0.05$). While PEVs from HNC patients promoted CD8+ T cell apoptosis and downregulated NKG2D on NK cells ($p<0.05$), SEVs mediated CD8+ T cell apoptosis to a lesser extent ($p<0.05$).

Conclusions: SEVs from HNC patients represent an easily accessible and well-detectable biomarker source, however, SEVs are less abundant and show less inhibitory effects in comparison to PEVs. To assess their potential as biomarkers more effectively, it is necessary to continuously monitor additional HNC-related proteins and their effects on other immune cells in a larger cohort of patients.

Epidemiology, Prevention and Diagnose

L2
Proof of concept study for HPV16 E6 serology based screening and early detection of HPV-driven oropharyngeal cancer

D. Höfler[1], A. S. Hoffmann[2], B. Becker[2], F. Rosing[1], F. Meyer[2], L. Schroeder[1], J. Butt[1], I. Schäfer[2], E. Petersen[2], C. J. Busch[3], C. Betz[2], T. Rieckmann[2], T. Waterboer[1]

[1]German Cancer Research Center (DKFZ), Infections and Cancer Epidemiology, Heidelberg, Germany
[2]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany
[3]Greifswald University Hospital, Greifswald, Germany

Objective: An increasing proportion of oropharyngeal cancers (OPC) are caused by human papillomavirus (HPV-OPC). Serum antibodies against HPV16 early proteins as well as cell-free HPV DNA (cfHPV DNA) in liquid biopsies are emerging pre-diagnostic markers. We present a prospective proof-of-concept study of HPV serology-based 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 digital PCR.

Results: Ten of 12 participants, considered to be at high-risk, participated in regular follow-up examinations. After 5 years of active follow-up, 5 of these 10 were diagnosed with stage I HPV-OPC. Additional 11 out of 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. Three of the overall 6 cases presented with either no or a single lymph node metastasis. CfHPV DNA was detectable at diagnosis in 5 cases at varying levels, but not in the only 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.

Epidemiology, Prevention and Diagnose

L3
Identification of potential mRNA-biomarker for molecular non-invasive OSCC diagnostic and therapy control

L. Hose[1], C. Tekin[1], B. Verwaaijen[2], R. Kim[1], F. Brasch[3], L. U. Scholtz[1], I. Todt[1], M. Schürmann[1]

[1]Klinikum Bielefeld, Department of Otolaryngology, Head and Neck Surgery, Bielefeld, Germany
[2]Klinikum Bielefeld, Department of laboratory medicine, microbiology and transfusion, Bielefeld, Germany
[3]Klinikum Bielefeld, Department of Pathology, Bielefeld, Germany

Head and neck tumors, particularly oral squamous cell carcinomas (OSCC), represent a significant health challenge worldwide. OSCC accounts for over 90% of all malignancies within the oral cavity and is the sixth most common cancer globally, with 300,000 new cases, annually. The current diagnostic gold standard for OSCC involves tissue biopsies followed by histopathological evaluation. Sampling tissue biopsies is invasive, anesthetics must be administered, and it can result in patient discomfort, delays in treatment, and potential complications such as infection. In recent years, there has been a growing interest in non-invasive diagnostic methods, for example via a liquid biopsy like saliva or cytology-based analysis e.g. derived from swabs. These approaches could provide equally reliable results without the associated risks, and elaborate sampling by a specialized doctor. In this project, we worked on the characterization of non-invasive oropharyngeal samples for molecular diagnostics. After development of a robust method for mRNA-isolation for downstream analysis, we have generated RNA- Seq data to perform identification of applicable biomarkers in swab material of OSCC patients. qPCR verification of these markers in different test groups and combinatorial analyses allow us to differentiate between tumor patients and healthy probands having different medical histories. With a combination of the genes *SFN*, *c-JUN* and *HSP90AB01*, we achieved a diagnostic accuracy of 0.91 (AUC). Interestingly, upregulation of some of these markers have already been associated with other cancer types. Further fluorescence staining of archived primary tissue confirmed tumor specificity at the protein level for OSCC. Herewith we show a way to identify diagnostic mRNA-based markers and were able to find potential markers for diagnostics, therapy control and new therapeutic approaches.

Immunotherapy, Cellular Therapy and Vaccination

L4
Marginal Zone B cells act as professional antigen-presenting cells in head and neck squamous cell carcinoma

J. Bao[1,2], J. Hess[3], S. Schmid[4], G. Berry[1], P. J. Schuler[1,3], J. Greve[1], S. Laban[1], T. K. Hoffmann[1], M. Mulaw[5], C. Brunner[1,6]

[1]University Ulm Medical Center, Department of Oto-Rhino-Laryngology, Ulm, Germany
[2]Southeast University Nanjing, School of Medicine, Nanjing, China
[3]Heidelberg University Hospital, Department of Oto-Rhino-Laryngology, Heidelberg, Germany
[4]University Ulm Medical Center, Department of Anesthesiology and Intensive Care Medicine, Ulm, Germany
[5]University Ulm, Unit for Single-Cell Genomics, Ulm, Germany
[6]University Ulm, Core Facility Immune Monitoring, Ulm, Germany

Background: Current data assign B lymphocytes a significant contribution to tumor development, including head and neck squamous cell carcinoma (HNSCC). Due to their high developmental diversity and different regulatory and functional roles, B cell subpopulations can promote or inhibit tumor growth. Therefore, the investigation of different B cell subpopulation within the microenvironment is of particular importance.

Methods: Using flow cytometry, we discovered the presence of marginal zone B cells (MZB) both within tumor tissue and peripheral blood in a cohort of HNSCC patients. To confirm these data and to get more insight into the possible function of tumor-associated MZB, we analyzed four publicly available single-cell RNA sequencing (scRNA-seq) datasets.

Results: Unsupervised clustering and reference-based cell annotation revealed two distinct MZB populations, characterized by their robust intercellular communication and anti-viral pathways. Cell communication analysis revealed abundant MHC-I and MHC-II antigen presentation between MZB clusters and various CD4+ and CD8+ T cell subpopulations. MZB-2 exhibit a higher degree of functional maturity, engage in more pronounced interactions with CD4+ T cells and antigen-presenting cells, and are of prognostic significance for HNSCC patients. A geographical proximity of MZB clusters and T cells was detected by spatial transcriptomics.

Conclusion: Our study unveiled the existence of two distinct subsets of MZB within the tumor and peripheral blood of HNSCC patients, with a pivotal role in antigen presentation and T cell activation. Harnessing the potential of these immunogenic B cells represents a promising therapeutic avenue of which HNSCC patients could benefit in the future.

Immunotherapy, Cellular Therapy and Vaccination

L5
Glutamine synthetase expression enhances T cell effector functions in a glutamine-restricted environment

S. Decking[1], I. Ugele[1], C. Bruss[2], C. Bohr[1], M. Kreutz[2], K. Renner[1]

[1]University Hospital Regensburg, Department of Otorhinolaryngology, Regensburg, Germany
[2]University Hospital Regensburg, Department of Internal Medicine III, Regensburg, Germany

Objective: T cells are highly dependent on glutamine to sustain metabolic activity and effector functions. Low glutamine levels within the tumour microenvironment have been associated with a diminished immune response, potentially affecting also the efficacy of adoptive T cell transfer therapies. We hypothesized, that expression of glutamine synthetase (GS), catalyzing the de-novo synthesis of glutamine from glutamate, could mitigate the effects of glutamine restriction on T cells.

Methods: Retroviral transduction was used to express the GS in human CD4+ T cells. GS-expressing cells were analysed for metabolic activity, proliferation and cytokine production under glutamine-restricted conditions. Furthermore, the anti-tumour activity of GS-expressing T cells was evaluated in co-cultures with HNSCC 3D tumour spheroids.

Results: Retroviral transduction resulted in strong and robust GS expression in human T cells. GS-expressing T cells displayed a restored respiratory and proliferative activity under glutamine-restricted conditions, in particular in the presence of glutamate. IFNγ secretion under glutamine limitation was elevated by GS expression. Of significant importance, upon co-cultivation with HNSCC spheroids GS-expressing T cells showed increased effector functions in the presence of low glutamine concentrations.

Conclusion: The results of our study indicate that GS expression represents a promising strategy to enhance anti-tumour effector functions, suggesting that metabolic engineering of T cells could be a potential strategy in cell therapy approaches.

Immunotherapy, Cellular Therapy and Vaccination

L6
The effect of HNSCC-derived exosomes on the immunological function of neutrophil granulocytes

A. Froschermaier[1], B. Schmidl[1], L. Griesbaum[1], A. B. Dezfouli[1], H. Mai[1], B. Wollenberg[1], M. Wirth[1]

[1]Klinikum rechts der Isar, Technical University Munich, Poliklinik für Hals-, Nasen-, Ohrenheilkunde, Munich, Germany

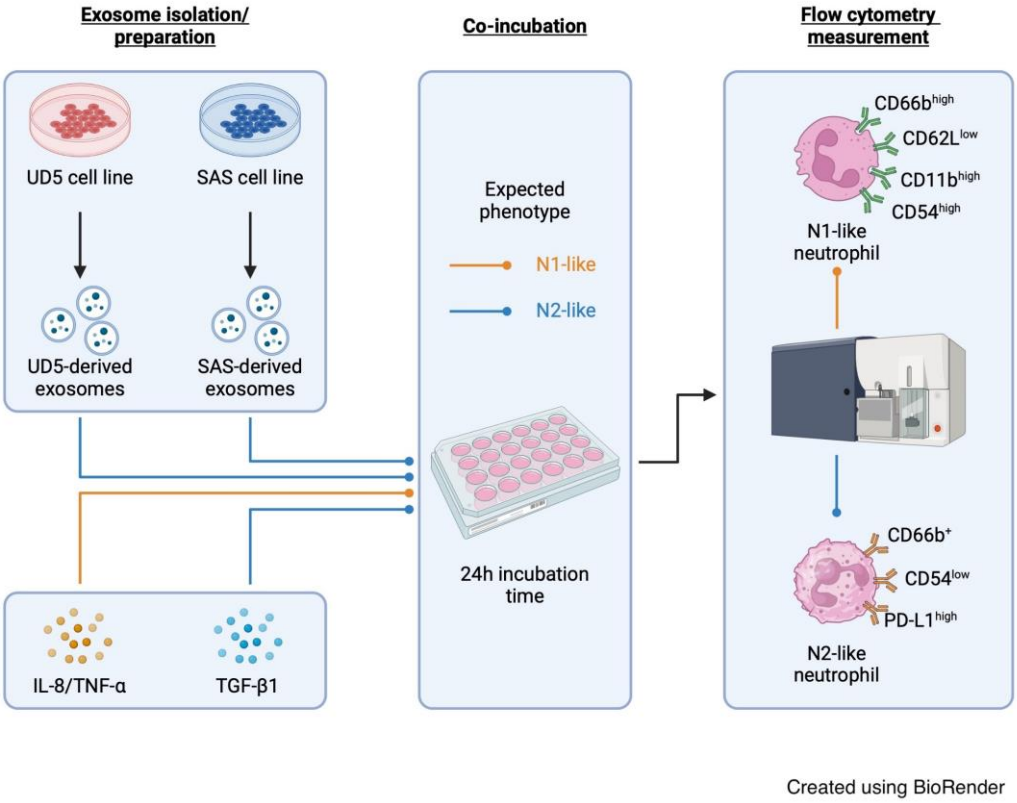
Objective: Neutrophil granulocytes are the most abundant immune cell type in the blood acting as a first line of immune defense. Recent findings show that neutrophils play an important role in the tumor microenvironment (TME), displaying an anti- or pro-tumor phenotype (N1-like / N2-like). This study aims to explore the effect of HNSCC-derived exosomes on neutrophil granulocytes to explore whether they are able to create an N2-like pro-tumor neutrophil phenotype.

Methods: Exosomes were isolated from the supernatant of two HNSCC cell lines (UD5, SAS) using size exclusion chromatography. Diameter and amount of exosomes were verified using a nanoparticle tracking video microscope. Venous blood was collected from healthy donors and neutrophils were isolated (n=4). The neutrophils were then co-incubated with cell-line-derived exosomes or two cytokine treatments (IL-8/TNF-α, TGF-β1) as a control. The stimulated neutrophils were harvested after a maximum of 24h co-culture and stained for cell surface proteins to investigate phenotypic changes using FACS.

Results: Neutrophils stimulated with IL-8 and TNF-α did show a $CD66b^{high}$, $CD62L^{low}$, $CD11b^{high}$, $CD54^{high}$ expression pattern, congruent with an N1-like phenotype. Neutrophils stimulated with TGF-β1 show a slight but not significant decrease in CD66b and no activation or degranulation. Neutrophils co-incubated with SAS- or UD5-derived exosomes show an insignificant decrease in CD66b and CD11b, the expression of PD-L1 was not increased.

Conclusion: Neutrophil granulocytes can be shifted into an N1-like phenotype using a combination of IL-8 and TNF-α in vitro. When stimulating neutrophils with TGF-β1, no clear N2-like phenotype could be established yet, raising the question if neutrophils can be shifted into an N2-like state by using this experimental setup. The co-incubation of neutrophils with HNSCC-derived exosomes doesn"t show significant marker shifts. To confirm whether these exosomes have an immunological effect on neutrophils, further experiments need to be conducted.

Fig. 1


Exosome isolation/ preparation
Co-incubation
Flow cytometry measurement
UD5 cell line
SAS cell line
UD5-derived exosomes
SAS-derived exosomes
IL-8/TNF-α
TGF-β1
Expected phenotype
N1-like
N2-like
24h incubation time
CD66b high
CD62L low
CD11b high
CD54 high
N1-like neutrophil
CD66b+
CD54 low
PD-L1 high
N2-like neutrophil
Created using BioRender

Interdisciplinary Tumor Board

L7
Molecular Insights into tumor budding in HNSCC: A multi-omics exploration

I. Ourailidis[1,2], F. Stögbauer[3], Y. Zhou[3], S. Beck[1], E. Romanovsky[1], S. Eckert[3], B. Wollenberg[3], M. Wirth[3], K. Steiger[3], B. Kuster[3], O. Gires[4], A. Stenzinger[1], P. Schirmacher[1], W. Weichert[3], P. H. Kuhn[5], M. Boxberg[3], J. Budczies[1]

[1]Heidelberg University Hospital, Institute of Pathology, Heidelberg, Germany
[2]University of Heidelberg, Faculty of Biosciences, Heidelberg, Germany
[3]Technical University of Munich, Munich, Germany
[4]Ludwig Maximilian University of Munich, Munich, Germany
[5]Institute of Pathology Kaufbeuren Memmingen Ravensburg, Kaufbeuren, Germany

Objective: Tumor budding (TB) is a key prognostic marker in HPV-negative HNSCC and is gaining attention in HPV-positive case[1]. Linked to partial epithelial-mesenchymal transition (EMT)[2], the molecular mechanisms of TB remain unclear. This study explores the molecular basis of TB in HNSCC using multi-omics approaches.

Methods: We analyzed TCGA data (mutations, miRNA, transcriptome, proteomics) and in-house proteomics and IHC data. Differential expression and pathway enrichment analyses were conducted to compare budding and non-budding tumors. Statistical significance and molecular overlaps were assessed across multiple datasets.

Results: *NSD1* mutations correlated with lower TB in HPV-negative HNSCC. We found 66 differentially expressed miRNAs, including the miR-200 family, and 3,052 and 360 differentially expressed genes (DEGs) in HPV-negative and HPV-positive HNSCC, respectively. EMT and myogenesis were highly enriched in overexpressed genes. Proteomics of HPV-negative tumors identified 88 differentially expressed proteins overlapping with DEGs. IHC showed increasing CAV1 and MMP14 expression from non-budding tumors to the bulk of the budding tumors to tumor buds in both HPV-negative and HPV-positive cohorts.

Conclusion: Our multi-omics analysis provides insights into TB"s molecular drivers, suggesting *NSD1*, miRNAs, and proteins like CAV1 and MMP14 as potential biomarkers for TB in HNSCC, supporting the development of targeted treatments.

[1] Stögbauer F et al., Br J Cancer. 2023, 128(12):2295.

[2] Grigore AD et al., J Clin Med. 2016, 29;5(5):51.

Interdisciplinary Tumor Board

L8
Functional kinome profiling for personalized treatment of head and neck cancer

M. Kriegs[1,2], T. Rieckmann[1,3], M. Christopeit[4], K. Rothkamm[1], C. Betz[3], H. B. Zech[3]

[1]University Medical Center Hamburg-Eppendorf (UKE), Department of Radiotherapy & Radiation Oncology, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf (UKE), UCCH Kinomics Core Facility, Hamburg, Germany
[3]University Medical Center Hamburg-Eppendorf (UKE), Department of Otorhinolaryngology, Hamburg, Germany
[4]University Medical Center Hamburg-Eppendorf (UKE), II. Department of Medicine, Hamburg, Germany

Objective: For the personalized use of kinase inhibitors functional assays harbour significant potential to identify alternative targets and to improve the predictive accuracy. In this study we tested the implementation of functional kinome profiling for the molecular treatment of HNSCC.

Methods: The study included patients with tumors of the head and neck which were presented in the molecular tumor board at the University Cancer Center Hamburg. Fresh frozen tissue was analysed using functional kinome profiling as described in Bussmann *et al.* 2021 (Int J Cancer. doi: 10.1002/ijc.33606). The results were presented at the tumor board and discussed together with available additional molecular data.

Results: Until now we finalized the analysis of three out of six patients. For two cases, we unveiled alternative targets, which had not been identified by other methods. In vitro testing of appropriate kinase inhibitors (saracatinib and dasatinib respectively) indicated a high potential of these drugs to significantly block aberrant tumor-associated kinase activity. Due to the poor general condition of the patients, the appropriate therapies could not be carried out. For the third case, it was confirmed that the targeting of an existing FGFR mutation by erdafitinib leads to a significant reduction in kinase activity. The patient is currently undergoing erdafitinib therapy. Staging is scheduled for the beginning of November.

Conclusion: The functional testing of kinase activity can add valuable information for the personalized molecular treatment of tumors of the head and neck which might lead to alternative targets, better prediction and additional arguments to implement intended therapies. The main challenges are the availability of samples and the feasibility of the derived therapies.

Interdisciplinary Tumor Board

L9
Tumor tissue slices – A powerful translational tool for precising head and neck cancer radiotherapy

H. B. Zech[1], T. Rieckmann[1,2], A. Böttcher[1], N. Möckelmann[3], K. Rothkamm[2], C. Betz[1]

[1]University Medical Center Hamburg-Eppendorf (UKE), Otorhinolaryngology, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf (UKE), Lab. for radiobiology and radiation oncology, Hamburg, Germany
[3]Marienkrankenhaus Hamburg, Hamburg, Germany

Objective: Patient-derived tumor slices offer the opportunity to study individual patient responses to (targeted) radiotherapy, with the potential to tailor precision radiotherapy protocols in future.

Methods: Fresh patient-derived HNSCC samples were sectioned into 400 mm slices and cultured on cell culture inserts. The slice cultures were then irradiated, either alone or in combination with inhibitors (=potential radiosensitizer). After 2 and 24 hours, the samples were fixed and frozen. DNA double strand breaks (DSBs) were analyzed by quantifying 53BP1 foci in nuclei co-stained with the SCC marker p63 using immuno-fluorescence microscopy. Radiation induced DNA damage was correlated with patient"s clinical outcome to radiation therapy, if possible.

Results: Tumor slices from over 50 patients were successfully cultured (success rate >95%, stable oxygenation and proliferation >72 hours).

Project 1: HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) samples (n=14) had significantly higher residual DSBs than HPV-negative ones (n=12) post-radiation (3 Gy: 4.9 vs. 1.2 foci/nucleus; $p < 0.0001$). Smokers showed lower residual DSBs than non-smokers (6.5 vs. 3.2 foci/nucleus; p=0.0105).

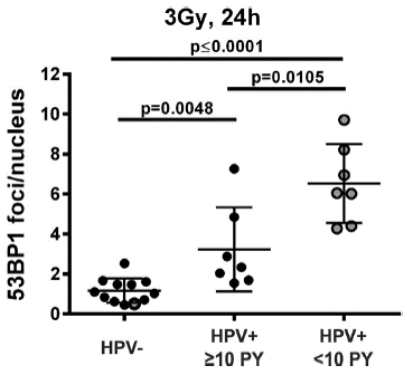
Project 2: Sinonasal squamous cell carcinoma (SNSCC) samples (n=16) showed variable residual DSBs linked to p16/HPV status. Higher ex vivo residual DSBs corresponded with favorable clinical radiotherapy response.

Project 3: WEE1/PARP inhibition significantly increased residual DSBs in HPV-negative OPSCC (n=5/7), not in HPV-positive samples (n=1/6), but also in SNSCC (n=4/6), indicating selective radiosensitization potential.

Conclusions: Ex vivo tumor slices provide a promising model for precision radiotherapy in head and neck cancer. Dual WEE1/PARP inhibition may enhance radiosensitivity in HPV-negative tumors, warranting further study.

Figure 1: Residual DSBs (=53BP1Foci) in HPV-negative vs. HPV positive tumors with different smoking history (py= pack years).

Fig. 1



Interdisciplinary Tumor Board

L10
De-escalation of adjuvant radio(chemo)therapy for patients with HPV-positive oropharyngeal squamous cell carcinoma (DELPHI study)

A. Linge[1,2,3,4], F. Lohaus[1,2,3,4], C. Rödel[5,6], S. Combs[7,8,9], A. L. Grosu[10,11], C. Belka[7,12,13], M. Stuschke[14,15], S. Böke[16,17], J. Debus[18,19,20,21,22], G. B. Baretton[2,3,23,24], S. Löck[1,2,3,4], M. Baumann[1,2,3,25], A. Abdollahi[18,19,20,21,26], M. Krause[1,2,3,4,27]

[1]OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Tec, Dresden, Germany
[2]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Dresden, Dresden, Germany
[3]National Center for Tumor Diseases (NCT), Partner Site Dresden, Dresden, Germany
[4]Technische Universität Dresden, Department of Radiotherapy and Radiation Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden, Germany
[5]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Frankfurt, Frankfurt, Germany
[6]Goethe-University Frankfurt, Department of Radiotherapy and Oncology, Frankfurt, Germany
[7]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Munich, Munich, Germany
[8]Technische Universität München, Department of RadioOncology, Munich, Germany
[9]Helmholtz Zentrum Munich, Department of Radiation Sciences (DRS), Institut für Innovative Radiotherapie (iRT), Munich, Germany
[10]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Freiburg, Freiburg, Germany
[11]University of Freiburg, Department of Radiation Oncology, Medical Center, Medical Faculty, Freiburg, Germany
[12]Ludwig-Maximilians-Universität, Department of Radiotherapy and Radiation Oncology, University Hospital, Munich, Germany
[13]Helmholtz Zentrum Munich, Clinical Cooperation Group Personalized Radiotherapy in Head and Neck Cancer, Munich, Germany
[14]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Essen, Essen, Germany
[15]University of Duisburg-Essen, Department of Radiotherapy, Medical Faculty, Essen, Germany
[16]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Tübingen, Tübingen, Germany
[17]University Hospital Tübingen, Department of Radiation Oncology, Tübingen, Germany
[18]German Cancer Research Center (DKFZ), Heidelberg, Germany, and German Cancer Consortium (DKTK), partner site Heidelberg, Heidelberg, Germany
[19]University of Heidelberg Medical School and German Cancer Research Center (DKFZ), Heidelberg Institute of Radiation Oncology (HIRO), National Center for Radiation Research in Oncology (NCRO), Heidelberg, Germany
[20]University of Heidelberg Medical School, Heidelberg Ion Therapy Center (HIT), Department of Radiation Oncology, Heidelberg, Germany
[21]University of Heidelberg Medical School and German Cancer Research Center (DKFZ), National Center for Tumor Diseases (NCT), Heidelberg, Germany
[22]University of Heidelberg Medical School and German Cancer Research Center (DKFZ), Clinical Cooperation Unit Radiation Oncology, Heidelberg, Germany
[23]Technische Universität Dresden, Institute of Pathology, Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden, Germany
[24]Technische Universität Dresden, Tumour- and Normal Tissue Bank, University Cancer Centre (UCC), University Hospital Carl Gustav Carus, Dresden, Germany
[25]German Cancer Research Center (DKFZ), Division of Radiooncology/Radiobiology, Heidelberg, Germany
[26]University of Heidelberg Medical School and German Cancer Research Center (DKFZ), Translational Radiation Oncology, Heidelberg, Germany
[27]Helmholtz-Zentrum Dresden - Rossendorf, Dresden, Germany

For patients with locally advanced head and neck squamous cell carcinoma (HNSCC) that are receiving resection of the primary tumour and neck dissection, adjuvant radio(chemo)therapy is prescribed according to risk factors, following the respective guidelines. However, it has been shown, that (I) patients with HPV-positive oropharyngeal tumours are showing very high tumour control rates after adjuvant radio(chemo)therapy, and (II) HPV-positive oropharyngeal squamous cell carcinomas (OPSCC) have an increased radiosensitivity. This suggests that the subgroup of patients with HPV-positive OPSCC is currently being overtreated. At the same time, patients are becoming long-term survivors but experiencing more chronic toxicity and reduced quality of life.

This prospective, multicentric, non-randomised phase I dose-deescalation study (DELPHI study) has been set up in patients with HPV-positive OPSCC aiming to show that dose de-escalation of adjuvant radiotherapy is safe and leads to less chronic toxicity and improved quality of life without compromising local tumour control. To date, locoregional recurrences in the de-escalation arms have not been detected.

Further details and the current status of the DELPHI study will be presented.

Radiooncology and Imaging

L11
Predicting immunometabolic status in head and neck squamous cell carcinomas using multiparametric PET/MRI: Study plan and preliminary validation of biomarkers

C. Kürten[1], I. Özel[1], P. Elahi[1], T. Hussain[2], S. Lang[1], J. Jablonska[1], B. Schaarschmidt[3]

[1]University Hospital Essen, Department of Otorhinolaryngology, Head and Neck Surgery, Essen, Germany
[2]Technical University of Munich, Department of Otorhinolaryngology, Head and Neck S, Munich, Germany
[3]University Hospital Essen, Department of Radiology, Essen, Germany

Question: Head and neck squamous cell carcinoma (HNSCC) poses a continued therapeutic challenge especially in advanced stages. While immunotherapy has shown efficacy in metastatic settings, its integration in curative treatments remains elusive, with only a small subset of patients benefiting. Non-invasive techniques like multiparametric PET/MRI offer the potential to predict tumor immunometabolic status, enabling better treatment selection. This study aims to correlate immunopathological characteristics of HNSCC with imaging biomarkers to improve patient stratification and therapeutic outcomes.

Methods: In this analysis, as an extension of the InterSCCede trial, 140 patients with HNSCC underwent 18F-FDG PET/MRI imaging prior to therapy. We aim to correlate immunometabolic profiles, using bulk RNA sequencing and multicolor immunohistochemistry (IHC), with imaging biomarkers such as ADC and SUV measurements. Extensive clinical follow-up and prospective data collection ensures a comprehensive cohort analysis. Immunopathological assessment focused on immune cell infiltration (CD8 effector T cells, Treg infiltration, Macrophage polarization and count, total neutrophil infiltration) while RNAseq investigates global gene set enrichment and pathways.

Results: Here we present the study plan as well as preliminary validation and quality control experiments for both IHC and RNA sequencing. Imaging biomarkers (ADC and SUV) are currently being correlated with immunopathological findings to assess their prognostic value.

Conclusions: The combination of immunometabolic profiling and multiparametric PET/MRI holds promise for improving HNSCC patient selection for immunotherapy. This approach could lead to more personalized treatment strategies and enhance survival outcomes by providing deeper insights into the tumor microenvironment and its role in therapy response.

L12
Tissue microarray analyses of the essential DNA repair factors ATM, DNA-PKcs and KU80 in head and neck squamous cell carcinoma

T. Rieckmann[1], C. M. von Bargen[2], A. Oetting[2], N. Möckelmann[3], N. Struve[2], C. Petersen[2], C. Betz[2], K. Rothkamm[2], A. Münscher[3], T. S. Clauditz[2], H. B. Zech[2]

[1]University Medical Center Hamburg-Eppendorf (UKE), Department of Otorhinolaryngology & Department of Radiotherapy, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany
[3]Marienkrankenhaus, Hamburg, Germany

Question: Head and neck squamous cell carcinoma (HNSCC) negative for Human Papillomavirus (HPV) has remained a difficult to treat entity, whereas tumors positive for HPV are characterized by radiosensitivity and favorable patient outcome. On the cellular level, radiosensitivity is largely governed by the tumor cells` ability to repair radiation-induced DNA double-strand breaks (DSBs), but no biomarker is established that could guide clinical decision making. Therefore, we tested the impact of the expression levels of ATM, the central kinase of the DNA damage response as well as DNA-PKcs and Ku80, two major factors in the main DSB repair pathway non-homologous end joining (NHEJ).

Methods: A tissue microarray of a single center HNSCC cohort was stained for ATM, DNA-PKcs and Ku80 and the expression scored based on staining intensity and the percentages of tumor cells stained. Scores were correlated with clinicopathological parameters and survival.

Results: Samples from 427 HNSCC patients yielded interpretable stainings and were scored following an established algorithm. The majority of tumors showed strong expression of both NHEJ factors, whereas the expression of ATM varied more. The expression scores of ATM and DNA-PKcs were not associated with patient survival. For HPV-negative HNSCC, the minority of tumors without strong Ku80 expression trended towards superior survival when treatment included radiotherapy. Focusing stronger on staining intensity to define the subgroup with lowest and therefore potentially insufficient expression levels in the HPV-negative subgroup, we observed significantly better overall survival for patients treated with radiotherapy but not with surgery alone.

Conclusions: Our data suggest that HPV-negative HNSCC with particularly low Ku80 expression represent a highly radiosensitive subpopulation. Confirmation in independent cohorts is required.

L13
CD44 transcript variants as determinants of HNSCC radioresistance: Metabolic implications and therapeutic vulnerabilities

U. Theiß[1,2], K. Gehr[1], M. Orth[1,3], M. Selmansberger[4], B. Dankó[4], F. Moritz[5], C. Müller[5], J. Maas[1], J. Hess[1,4], K. Unger[1,4,6], H. Zitzelsberger[1,4], C. Belka[1,6,7], K. Lauber[1,7]

[1]Ludwig Maximilian University of Munich, Radiation Oncology, Munich, Germany
[2]Philipps-University Marburg, Radiotherapy and Radiation Oncology, Marburg, Germany
[3]University Hospital Tübingen, Radiation Oncology, Tübingen, Germany
[4]Helmholtz Center Munich, Research Unit Radiation Cytogenetics, Neuherberg, Germany
[5]Helmholtz Center Munich, Analytical Biogeochemistry, Neuherberg, Germany
[6]Bavarian Center for Cancer Research (BZKF), Munich, Germany
[7]German Cancer Consortium (DKTK), Munich, Germany

Objective: 5-year survival rates for locally advanced HNSCC remain poor, with cellular radioresistance posing a major obstacle. This study focused on the cell surface marker CD44, which has been implicated in mediating radio- and chemoresistance across various cancer entities, including HNSCC. The impact of CD44 on radioresistance was systematically evaluated at the level of individual transcript isoforms through in vitro and in vivo models, and in clinical samples.

Methods: Isoform-specific analysis of CD44v transcript variants was conducted in two independent patient cohorts. In vitro, clonogenic survival, cell fate decisions, gene expression profiling, cytokine response, and metabolic profiling were assessed in seven irradiated HNSCC cell lines with varying basal or siRNA-silenced CD44v expression levels. In vivo, the impact of CD44v expression on radiotherapeutic outcomes was evaluated using an orthotopic HNSCC mouse model. The mechanisms underlying these observations are currently under investigation.

Results: HNSCC patient data revealed significant negative associations between CD44v expression and overall survival, as well as other clinical endpoints. Consistently, CD44v transcript levels were positively correlated with in vitro radioresistance across a panel of HNSCC cell lines. Isoform-specific knockdown led to increased basal levels of senescence, enhanced cell death upon irradiation, and reduced clonogenic survival. Notably, CD44v silencing induced large-scale metabolic alterations and lipid peroxidation, which were further exacerbated by irradiation. In vivo, CD44v-silenced orthotopic HNSCC xenotransplants showed signigicantly improved outcomes upon radiotherapy.

Discussion: Our study identifies CD44v transcript isoforms and their associated metabolic functions as critical mediators of radioresistance in HNSCC, highlighting them as both promising prognostic biomarkers and potential therapeutic targets for improving treatment outcomes in HNSCC.

Molecular Landscape, Multiomics and Artificial Intelligence

L14
Personalized cell-free circulating tumour DNA analysis for patients with HNSCC

S. Flach[1,2], K. Howarth[3], C. Pipinikas[4], S. Hackinger[4], T. Huberty[1], A. Lechner[1], G. Marsico[4], C. Walz[5], L. Käsmann[6,2], P. Jurmeister[5,2], S. Stöcklein[7], G. Abaci[7], C. A. Reichel[1], O. Gires[1], M. Canis[1], P. Baumeister[1]

[1]LMU Klinikum München, Otorhinolaryngology, Head & Neck Surgery, Munich, Germany
[2]German Cancer Consortium (DKTK), Partner Site München, Munich, Germany
[3]Bradfield Centre, Cambridge, United Kingdom
[4]NeoGenomics, Cambridge, United Kingdom
[5]Ludwig Maximilian University of Munich, Institute of Pathology, Munich, Germany
[6]LMU Klinikum München, Radiotherapy and Radiation Oncology, Munich, Germany
[7]LMU Klinikum München, Radiology, Munich, Germany

Objective: Early relapse and development of metastatic disease are some of the primary reasons for the poor prognosis of HNSCC. We conducted a prospective cohort study to assess ctDNA in plasma and saliva from patients with HNSCC receiving primary surgery with curative intent. Objectives were to determine whether postoperative ctDNA detection can act as biomarker for surgical tumour clearance and to evaluate the potential of tumour-informed ctDNA analysis for early molecular-level detection of relapse. Furthermore, we investigated the molecular and clinical features associated with ctDNA release in HNSCC.

Methods: Plasma and saliva samples from 76 HNSCC patients were collected pre- and postoperatively and during follow-up. Whole exome sequencing was performed on FFPE tumour tissue. Tumour-specific variants for personalized assay design were selected and used in the analysis of serial samples for evidence of MRD. ctDNA levels were correlated to tumour volumes from staging CT, as well as pathological and other clinical parameters. RNA sequencing was conducted on 58 primary tumour samples and transcriptional profiles were correlated to ctDNA shedding.

Results: 617 longitudinal plasma and 128 saliva samples were collected and analyzed. Increased plasma ctDNA levels were detected postoperatively in 96% of cases with confirmed clinical recurrences (21/22) with a median lead time of 160 days. ctDNA was detected in baseline saliva samples from patients with HNSCC of various anatomical locations with a 69% overlap with the corresponding plasma ctDNA profiles. Pathological tumour stage, lymph node involvement as well as transcriptional pathways associated with proliferation were strongly correlated with preoperative ctDNA shedding.

Conclusion: The use of ctDNA detection in surgically treated patients with HNSCC has significant potential to guide treatment decisions, improve disease outcome and potentially spare patients invasive interventions during follow-up.

Molecular Landscape, Multiomics and Artificial Intelligence

L15
Single-cell spatial analysis of the salivary gland carcinoma microenvironment

C. Arolt[1], L. Jansen[2], J. P. Klußmann[2], A. Quaas[1], L. Nachtsheim[2], M. Mayer[2]

[1]University of Cologne, Institute of Pathology, Cologne, Germany
[2]University of Cologne, Department of Otorhinolaryngology, Head and Neck Surgery, Cologne, Germany

Objective: To correlate the single-cell tumor microenvironment (TME) of non-myoepithelial salivary gland carcinomas (nonMYO SGC) with patient outcome to evaluate new prognostic biomarkers.

Methods: A cohort of 54 SGC was analyzed with a 13-marker imaging mass cytometry antibody panel using a Hyperion-CyToF System including primaries and nodal metastases. From multichannel image data, we generated a 13-plex single cell data set of tumor cells, cancer associated fibroblasts (CAFs), and immune cells. Cell phenotyping using an established CAF-classification algorithm, spatial analysis and correlation of the cellular landscape with clinicopathological features were carried out.

Results: After filtering, 509,364 cells from nonMYO SGC (i.e., salivary duct carcinomas (SDC), acinic cell, mucoepidermoid, and secretory carcinomas) were assigned to one of 15 cell phenotypes using multistep clustering. We observed that SDC exhibited the highest fraction of Collagen-CAFs and significantly elevated Matrix-CAFs (mCAFs; $p < 0.05$). Acinic cell carcinomas were enriched for CD4+Tcells ($p < 0.05$), CD8+Tcells ($p < 0.05$), and antigen-presenting CAFs ($p < 0.1$). Also, a spatially defined cellular neighborhood of mCAFs and endothelia (CN8) was significantly elevated in SDC ($p < 0.05$). Analyses evaluating the prognostic impact were carried out in SDC ($n = 23$) after dichotomizing them based on cellular and CN frequencies. Higher frequencies of mCAFs and the associated CN8 were significantly associated with a reduced recurrent-free survival and distant control rate ($p < 0.05$). SDC with a higher fraction of CD8+Tcells showed a trend for a reduced distant control rate ($p < 0.1$).

Conclusion: NonMYO SGC entities display specific TME cell compositions. In SDC, mCAFs show a significant impact on prognosis.

Molecular Landscape, Multiomics and Artificial Intelligence

L16
Molecular landscape of oral cancer in young adults

E. Kolegova[1], M. Patysheva[1], E. Prostakishina[1], A. Korobeynikova[1], M. Menyailo[1], R. Vorobev[1], V. Korobeynikov[1], I. Fedorova[2], D. Kulbakin[2], A. Mordovsky[3], D. Kudashkina[3], A. Polyakov[3], A. Kaprin[3], A. Vyalov[4], L. Yakovleva[4], V. Tsiklauri[5], O. Saprina[5], M. Kropotov[5], N. Sukortseva[6], S. Samoilova[6], I. Reshetov[6], E. Choinzonov[2], E. Denisov[1]

[1]Cancer Research Institute, Tomsk National Research Medical Center of Russian Academy of Sciences, Laboratory of Cancer Progression Biology, Tomsk, Russian Federation
[2]Cancer Research Institute, Tomsk National Research Medical Center of Russian Academy of Sciences, Department of Head and Neck Tumors, Tomsk, Russian Federation
[3]P.A. Herzen Moscow Oncology Research Institute – a branch of the National Medical Research Radiological Center, Microsurgery Department, Moscow, Russian Federation
[4]A.S. Loginov Moscow Clinical Scientific Center, Department of Head and Neck Tumors, Moscow, Russian Federation
[5]N.N. Blokhin National Medical Research Radiological Center, Surgical Department N10 of Head and Neck Tumors, Moscow, Russian Federation
[6]Radiotherapy and Plastic Surgery, I.M. Sechenov First Moscow State Medical University, Department of Oncology, Moscow, Russian Federation

Objective: Oral carcinoma (OC) is characterized by rapid disease progression. The incidence of OC among individuals under 45 years of age is growing every year. But, the pathogenetic mechanisms of early-onset OC are poorly understood. The aim of the study was to investigate molecular features of OC in young adults (<45 years).

Method: The study included 41 patients with T1-4N0-2M0 OC divided into two groups: younger (n=28) and older than 45 years (n=13). Peripheral blood, freshly frozen tumor and formalin-fixed paraffin-embedded tumor samples were used to perform whole-exome, 16S rRNA, single cell and spatial transcriptomics sequencing.

Results: Mutational landscape of early-onset OC was characterized by increase in *LOC112267881* and exon 8 of the *TP53* mutation and *DEFB125* amplification frequency. Functional annotation of other genes whose mutations occurred only in young adults revealed significant enrichment of Rap1 and MAPK signaling pathways. According to the single cell and spatial transcriptomics results, tumor clusters in young adults showed a downregulation of genes associated with oxidative phosphorylation and upregulation of genes associated with the MAPK and JAK-STAT signaling pathways and vascular mimicry. The microenvironment of early-onset OC was enriched with tumor-associated macrophages in regions of vascular mimicry. Based on 16S rRNA sequencing, a significant enrichment of *Acidovorax caeni, Streptococcus infantis*, and *Treponema amylovorum* and depletion of *Bacteroides acidifaciens, Bibersteinia trehalosi, Kocuria rhizophila*, and *Pseudomonas veronii* in OC of young adults was found compared to patients older than 45 years.

Conclusion: Early-onset OC demonstrates the specific mutational, transcriptome, microenvironmental, and microbiome landscapes and can be distinct molecular entity requiring the individualized treatment strategy.

This work was supported by the Russian Science Foundation (project № 22-15-00308).

Mechanisms of Treatment Failure and New Drug Targets

L17
EGFR-mediated local invasiveness and response to cetuximab in head and neck cancer

O. Gires[1]

[1]Klinik und Poliklinik für Hals-Nasen-Ohrenheilkunde, HNO-Forschung, Munich, Germany

Background: Recurrent/metastatic head and neck squamous cell carcinoma (R/M-HNSCC) is a severe, frequently lethal condition. Oncogene addiction to epidermal growth factor receptor (EGFR) is a hallmark of HNSCC, but clinical efficacy of EGFR-targeted therapies remains low. Understanding molecular networks governing EGFR-driven progression is paramount to the exploration of (co)-treatment targets and predictive markers.

Methods: We performed function-based mapping of differentially expressed genes in EGFR-mediated local invasion (fDEGs) using photoconvertible tracers and RNA-sequencing (RNA-seq) in a cellular 3D-model.

Results: Upon alignment with single-cell RNA-seq (scRNA-seq) and HNSCC-specific regulons, a gene regulatory network of local invasion (invGRN) was inferred from gene expression data. InvGRN comprises the central hubs inhibin subunit beta alpha (*INHBA*) and snail family transcriptional repressor 2 (*SNAI2*), and druggable fDEGs integrin subunit beta 4 (*ITGB4*), laminin 5 (*LAMB3*/*LAMC2*), and sphingosine kinase 1 (*SPHK1*). INHBA inhibition repressed local invasion and was reverted by activin A, laminin 5, and sphingosine-1-phosphate, demonstrating a functional interconnectivity of the invGRN, which was overrepresented in budding tumors. fDEGs and epithelial-to-mesenchymal transition (EMT) of malignant cells are induced by newly defined EGFR-activity subtypes with prognostic value that are promoted by amphiregulin (*AREG*) and epiregulin (*EREG*). Importantly, co-inhibition of SPHK1 showed synthetic effects on Cetuximab-mediated blockade of invasion and high expression of selected fDEGs was associated with response to Cetuximab in patient-derived xenotransplantation (PDX) and R/M-HNSCC patients.

Conclusions: We describe an actionable network of EGFR-mediated local invasion and define druggable effectors with predictive potential regarding the response of R/M-HNSCC to Cetuximab.

Mechanisms of Treatment Failure and New Drug Targets

L18
Mechanistic study of CCDC86 promoting the development of oral squamous cell carcinoma by regulating ribosome biogenesis

F. He[1,2], S. Na[1], J. Qiu[2]

[1]College of Stomatology, Xi'an Jiaotong University, Department of Oral and Maxillofacial Surgery, Xi'an, China
[2]The First Affiliated Hospital of Nanchang University, Nanchang, China

Background: Oral squamous cell carcinoma (OSCC) is a significant global health issue, with over 300,000 new cases annually, particularly in low- and middle-income countries. Major risk factors include tobacco use, alcohol consumption, HPV infection, and poor oral hygiene. Despite advances in diagnostic and therapeutic strategies, the 5-year survival rate for OSCC remains at approximately 50%. Ribosome biogenesis is essential for cellular proliferation and is often upregulated in cancer, including oral squamous cell carcinoma (OSCC), promoting tumor growth. However, the specific role of Coiled-Coil Domain Containing 86 (CCDC86) in OSCC, particularly its involvement in ribosome biogenesis, is not well understood.

Objective: This study aims to investigate the role of CCDC86 in OSCC progression, with a focus on its regulation of ribosome biogenesis and its interaction with nucleolin (NCL).

Methods: CCDC86 expression and function was analyzed in OSCC from TCGA-HNSC, clinical samples, cell lines with CCDC86 overexpression or knockdown, and in vivo xenograft models. Ribosome biogenesis and the interaction between CCDC86 and NCL were studied using biochemical assays and proteomics, and potential small-molecule inhibitors were identified.

Results: CCDC86 was significantly overexpressed in OSCC and correlated with poor prognosis. CCDC86 was positively associated with tumor cell migration, proliferation, invasion, while being inversely correlated with apoptosis. It promoted ribosome biogenesis by interacting with NCL and enhancing its lactylation at lysine residues 387 and 437. Two small molecules, ZINC4288612 and ZINC6095504, were identified as potential inhibitors of CCDC86-driven ribosome biogenesis and as potential therapeutic agents for OSCC.

Conclusion: CCDC86 plays a critical role in OSCC by regulating ribosome biogenesis and tumor progression, offering a promising therapeutic target.

Keywords: OSCC, CCDC86, ribosome biogenesis, nucleolin, lactylation.

Mechanisms of Treatment Failure and New Drug Targets

L19
Combined treatment of HPV-positive HNSCC cells with xevinapant, tuvusertib and ionizing radiation

N. H. N. Wahrhausen[1,2], A. Vahedi[1,2], A. Perugachi Heinsohn[1,2], F. Gatzemeier[1,2], J. Röhrle[2], C. Petersen[1], C. Betz[2], K. Rothkamm[1], T. Rieckmann[1,2]

[1]University Medical Center Hamburg-Eppendorf (UKE), Department for Radiotherapy and Radiation Oncology, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf (UKE), Department of Otorhinolaryngology, Hamburg, Germany

Question: A combination of the pro-apoptotic SMAC mimetic xevinapant and radiochemotherapy (RCT) demonstrated superiority over RCT alone in a phase 2 study in HNSCC, but the following phase 3 study in HPV-negative HNSCC was recently abrogated because it will not meet its primary endpoint. Regarding HPV-positive disease, clinical and preclinical data on xevinapant in combination with radiation are sparse. We tested the radiosensitization of HPV-positive HNSCC cells through xevinapant alone, as well as in combination with another emerging substance, the ATR-inhibitor tuvusertib.

Methods: Effectiveness of ATR-inhibition was tested through the potential to inhibit radiation-induced G2 cell-cycle arrest. Cytostatic effects were assessed by proliferation assay, radiosensitization by colony formation assay and apoptosis induction by testing for active caspase 3.

Results: While low doses of tuvusertib alone had little impact on proliferation and cell cycle distribution, they inhibited radiation-induced G2-arrest and conferred profound radiosensitization in all 4 HPV+ HNSCC cell lines tested so far. Sensitivity towards xevinapant varied with different strains demonstrating resistance, moderate radiosensitization but also profound sensitivity already under sole inhibitor treatment, indicating the need for a predictive marker. Sensitivity and radiosensitization were not necessarily accompanied by caspase 3 activity. Combined treatment with both inhibitors conferred substantial toxicity and/or radiosensitization in all HPV-positive cell lines tested so far.

Conclusions: In summary, sensitivity of HPV+ HNSCC cells towards xevinapant varies and prospective biomarkers or tests would be desirable. ATR-inhibition through tuvusertib resulted in profound radiosensitization and the combination of both agents appears promising. Mechanisms of cell death induction through xevinapant and combinations are further being investigated.

L20
Patients-derived (PD) cultures as preclinical models for tumor microenvironment

J. Dudas[1], M. D. C. Greier[1], J. Federspiel[1], C. Seifahrt[2], B. Hofauer[1]

[1]Medical University of Innsbruck, Department of Otorhinolaryngology and Head and Neck Surgery, Innsbruck, Austria
[2]Medical University of Innsbruck, Institute of Clinical-Functional Anatomy, Innsbruck, Austria

Question: The tumor microenvironment (TME) determines the reaction of head and neck squamous cell carcinoma (HNSCC) on given therapy and is in dynamic changes. We focus on two main TME components, the carcinoma-associated fibroblasts (CAFs) and the local immune microenvironment.

Methods: CAFs were isolated from biopsies and cultured in DMEM/F12. Stabilized and passaged CAFs were characterized by flow cytometry. SCC-25 HNSCC cells were added to semi-confluent fibroblasts culture.

Biopsy slices were cultured in Keratinocyte Medium, and were treated with T Cell Activation/Expansion Kit or with Pembrolizumab. After further culture the slices were fixed, paraffin embedded, sectioned and immunohistochemically stained for Granzyme B and Cleaved-Caspase-3 (CC3).

Results: CAFs were positive for podoplanin, alpha smooth muscle actin and fibroblast activation protein alpha. CAFs induced increased proliferation of the co-cultured SCC-25 cells, but also built physical barriers against the cancer cell nests, which induced stress-related signaling pathways.

Cultured tissue slices preserved their original architecture without loss of tumor or stroma cells. The local immune system was maintained, the immunohistochemical signals of Granzyme B and / or CC3 were patient-dependently changed by the treatments.

Conclusions: CAFs combined with tumor cell line culture display the influence of CAFs on tumor cells. PD tumor slices as "avatars" of the original tissue are excellent preclinical models with active local immune system and represent the immune microenvironment. Both models maintain patient characteristics.

New Technologies and Preclinical Models

L21
DARPin induced reactivation of p53 and its therapeutic potential for treatment of HPV induced tumors

P. Philipp Münick[1], B. Yüksel[1], M. Hufbauer[2], V. Dötsch[1], B. Akgül[2]

[1]Johann Wolfgang Goethe-Universität, Institute of Biophysical Chemistry, Frankfurt, Germany
[2]University of Cologne, Institute of Virology, Cologne, Germany

The infection of cells with high-risk (hr) HPV types causes cancer at various anatomical sites. Tumorigenesis is based on inactivation of key cellular control mechanisms by viral E6 and E7 proteins. The hrHPV E6 protein interacts with the cellular E3 ligase E6AP, and this complex binds to the p53 DNA-binding domain, resulting in ubiquitin-dependent degradation of p53. The inhibition of this interaction has the potential to reactivate p53, thereby preventing oncogenic transformation.

Using Ribosome display of a designed ankyrin repeat protein (DARPin) library, we identified a DARPin (C10) that binds to the same site as E6 in p53, thereby displacing E3 ligase and stabilizing p53. Subsequent biochemical analyses were performed to characterise the effects of DARPin C10 on cell physiology.

Interaction with DARPin C10 did not affect the DNA binding of p53 and reactivated a p53-dependent transcriptional program in HeLa and SiHa cells, resulting in reduced cell viability. We further showed that DARPin C10 does not interfere with the crucial MDM2-based regulatory mechanism of p53 in non-HPV-transformed cells. The affinity of DARPin C10 for mouse p53 is significantly lower than that for human p53. Based on this observation, we will describe an outline for future preclinical studies in immunodeficient and immunologically humanized mice by inducing xenograft tumors from HPV-positive cancer cell lines. Mice will be treated with mRNA/lipid-nanoparticles coding for DARPin C10, and tested for their ability to restore p53 and induce an anti-tumor effect either alone or in combination with cisplatin.

The preclinical data may serve as a basis to subsequently confirm DARPin C10 mRNA/lipid nanoparticles as a novel therapeutic approach for HPV-related malignancies in clinical trials, either alone or in the context of a radio-chemotherapy. The advantage of DARPin C10 is that it may block the binding of all hrHPV E6 proteins to p53.

New Technologies and Preclinical Models

L22
Label-free histopathology using SRS microscopy and AI for head and neck cancer evaluation

D. Pertzborn[1], T. Azevedo[2], E. Erriquez[3], F. Crisafi[3], E. Fantuzzi[3], M. Vali[2], R. Vanna[4], A. Mühlig[1], N. Ziller[1], A. C. Ferrari[2], P. Lio[2], G. Cerullo[5], M. Negro[3], O. Guntinas-Lichius[1]

[1]Jena University Hospital, Jena, Germany
[2]Univ. of Cambridge, Cambridge, United Kingdom
[3]Cambridge Raman Imaging S.r.l., Milan, Italy
[4]CNR-Istituto di Fotonica e Nanotecnologie, Milan, Italy
[5]Politecnico di Milano, Milan, Italy

Objective: Histopathological assessment of chemically stained, formalin-fixed, and paraffin-embedded tissue samples is the gold standard for evaluating head and neck cancer surgery outcomes. However, this process is time-consuming, requires chemical reagents, and demands personnel expertise. This study introduces Stimulated Raman Scattering (SRS) microscopy combined with AI as a label-free alternative to traditional chemical staining for evaluating head and neck cancer biopsies and resected specimens.

Methods: We combined SRS microscopy with machine learning to replicate chemical staining results. SRS imaging provides sub-cellular spatial resolution and molecular-specific contrast by detecting intrinsic vibrational fingerprints of cells and tissues. Unlike previous studies, we measured the entire CH spectrum (2800-3100 cm^{-1}) across 38 spectral channels at 1 µm resolution. Measurements were performed on routine pathology samples without additional preprocessing. Machine learning produced virtually stained slides for pathologist evaluation and directly performed diagnostic tasks, such as tumor segmentation.

Results: SRS microscopy with machine learning produced virtually stained slides nearly indistinguishable from chemically stained ones. We demonstrated machine learning-based tumor segmentation on SRS images with a Dice score >0.75. The total measurement and evaluation time for a typical slide was approximately 15 minutes.

Conclusion: This approach highlights a path to reducing reliance on time- and resource-intensive chemical staining for histopathological evaluation. The label-free nature of this workflow allows multiple virtual stainings and additional chemical processing on the same sample if needed.

Figure 1: Comparison of false color image created from SRS microscopy (left) and H&E staining (right) of the same head and neck tumor sample.

Figure 2: Comparison of tumor annotations by the pathologist (left) and the machine learning model (right).

Fig. 1

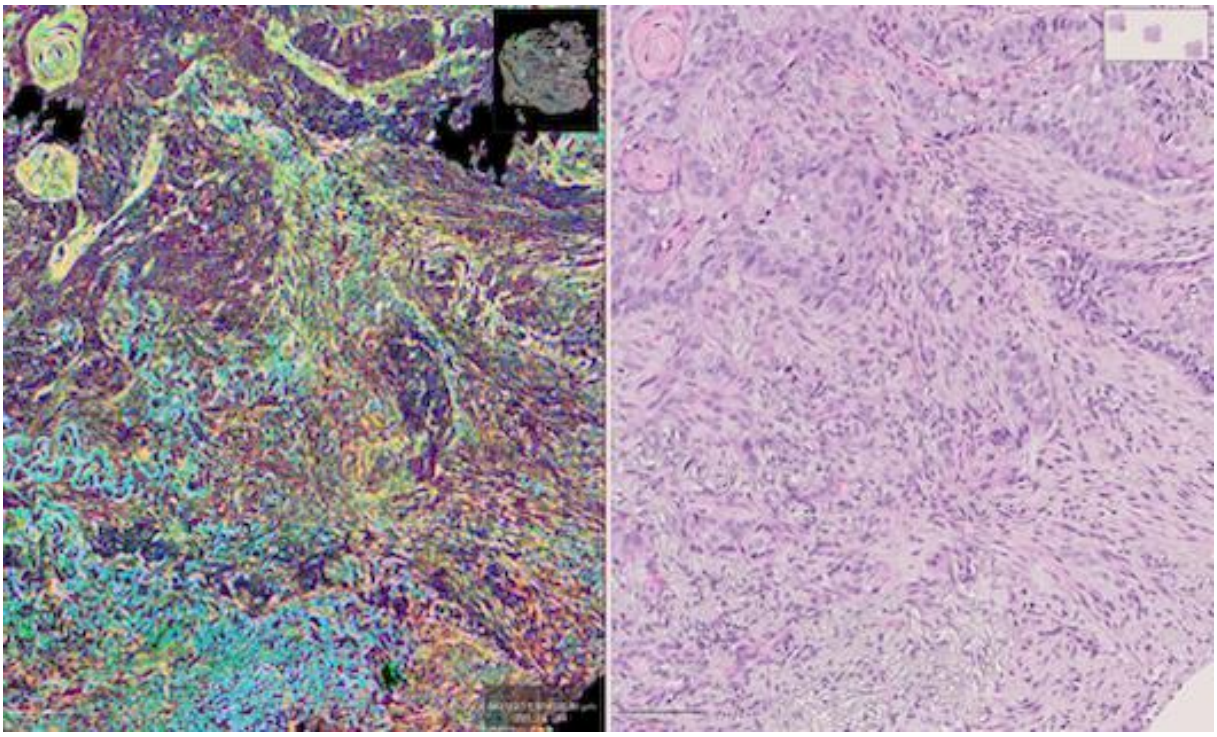
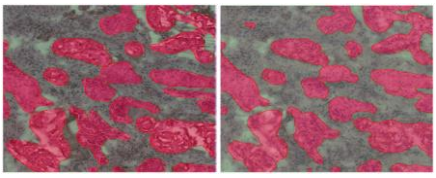


Fig. 2



Poster Session

P1
Mitochondrial gene signatures in the sex-specific pathogenesis and therapy of head and neck tumors

F. Döring[1], L. Fritz[1], E. Klostermann[1], J. Hess[1]

[1]university, Heidelberg, Germany

Background: Recent studies have shown a critical role of mitochondrial metabolic plasticity in head and neck squamous cell carcinoma (HNSCC) metastasis and sex-specific differences in mitochondria-related gene expression.

Objective: The aim of this study was to develop sex-specific prognostic risk models based on mitochondria-related gene expression. Cellular and molecular characteristics of the risk groups should be used to identify vulnerabilities for new treatment options.

Methods: The clinical and molecular data of TCGA-HNSC were used to define subgroups based on the mitochondria-related gene expression by unsupervised hierarchical clustering and to investigate their association with clinical variables by cross-tabulation. Sex-specific prognostic risk models were trained using LASSO penalized Cox regression and validated by multivariate Cox regression analysis. Differences in oncogenic gene sets and pathway activities between prognostic risk groups were explored by gene set variation analysis (GSVA) and oncoPredict was applied to infer potential vulnerabilities.

Results: LASSO penalized Cox regression revealed a prognostic risk model based on a mitochondria-related 501-gene set for men and disease-specific survival as a clinical endpoint. Significant differences were observed with respect to clinical variables, such as tumor size and lymph node involvement. Tumors of the high-risk group showed increased expression of gene sets related to DNA repair, MYC targets and oxidative phosphorylation, and oncoPredict predicted a higher sensitivity of these tumors to drugs targeting the ERK/MAPK pathway or DNA replication.

Conclusion: Our study confirms the potential of mitochondria-related gene signatures for sex-specific risk stratification of primary HNSCC. The results presented pave the way to explore the underlying molecular principles in preclinical models.

Poster Session

P2
Tracking tumor heterogeneity in head and neck squamous cell carcinoma (HNSCC)

Z. M. Uzun[1], J. George[1], J. P. Klußmann[1], O. Siefer[1], C. Müller[1]

[1]University of Cologne, HNO, Cologne, Germany

Objective: Head and Neck squamous cell carcinoma (HNSCC), the sixth most common cancer worldwide, exhibits high intra-tumor heterogeneity and clonal diversity. It is classified into HPV-positive, which typically has a better prognosis, and HPV-negative, associated with tobacco and alcohol use, displaying more aggressive behavior and poorer outcomes. We aim to study tumor heterogeneity in HNSCC across different tumor sites and over the course of treatment and to identify molecular mechanisms driving tumor progression and treatment resistance.

Methods: We will first analyze intra-tumoral heterogeneity at single time points referring to our own cohort of patients and to data acquired as part of the International Cancer Genome Consortium (ICGC). Additionally, we have gathered a multi-regional and longitudinal cohort from over 50 patients to investigate tumor evolution across different sites and under treatment. Bulk whole genome and exome sequencing (WGS) will be conducted, alongside the development of methods for single-cell genome and transcriptome sequencing of spatially and temporally matched tumor samples. Computational tools will be used to map clonal compositions and evolutionary trajectories across tumor sites.

Results: Analyses of single tumor sites using bulk genome sequencing data reveal significant intra-tumor heterogeneity. Our initial results from patient-matched pre-therapy and relapse tumor sites suggest branched evolutionary patterns. As we expand our analysis to a larger patient cohort, we expect to create a detailed, high-resolution map of tumor subclones, capturing their genetic evolution across different tumor sites and over time, resulting in a comprehensive multi-regional and longitudinal profile of HNSCC.

Conclusion: The study is expected to reveal key molecular mechanisms that drive HNSCC progression and resistance to treatment. Insights gained from tracking clonal dynamics will potentially lead to new approaches for tailoring patient-specific treatments.

Poster Session

P3
The mitochondrial plasticity in the pathogenesis and therapy of HPV16-negative head and neck tumors

L. Fritz[1], F. Döring[1], E. Klostermann[1], J. Hess[1]

[1]University of Heidelberg, Heidelberg, Germany

Background: Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies worldwide, significantly contributing to cancer-related morbidity and mortality. Recent studies have highlighted the crucial role of metabolic plasticity, particularly mitochondrial function, in driving metastasis.

Objective: The main objectives of this study were to provide a molecular classification of HNSCC and to establish a prognostic risk model based on the expression of a nuclear encoded gene set related to mitochondrial proteins with the ultimate goal of identifying promising drug targets and improving treatment outcome.

Results: Unsupervised hierarchical clustering of tumors from TCGA-HNSC (n=520), based on mitochondrial gene expression, revealed two main clusters (A and B), with B divided into subclusters (B1 and B2). These clusters showed significant clinical differences, including tumor subsite, HPV16 status, sex, and survival outcomes. A LASSO-penalised Cox regression analysis using disease-specific survival (DSS) as the clinical endpoint identified a prognostic mitochondrial-related gene set (n=17) for HPV16-negative tumors. The risk model was robust across different HNSCC subgroups, particularly in men and smokers, and showed prognostic relevance in other solid tumors from TCGA, demonstrating its broader clinical potential. Molecular analysis of low- and high-risk tumors revealed differences in copy number variations and pathways activities linked to cell cycle regulation, DNA repair, and energy metabolism. Finally, OncoPredict identified promising drug targets for high-risk tumors.

Conclusions: This study underscores the critical role of mitochondrial plasticity in HNSCC and other cancers. A prognostic risk model based on mitochondrial genes could guide new therapies targeting specific metabolic vulnerabilities.

Poster Session

P4
Silencing PTBP1 reduces HNSCC cell line proliferation

E. Schmidt Lorang[1], J. Mergner[2], A. Bashiri Dezfouli[1,3], M. Shoykhet[1], B. Wollenberg[1]

[1]Technical University of Munich, Department of Otolaryngology, Head and Neck Surgery, Munich, Germany
[2]Technical University of Munich, Bavarian Center for Biomolecular Mass Spectrometry, Munich, Germany
[3]Technical University of Munich, Central Institute for Translational Cancer Research, Munich, Germany

Objective: RNA-binding proteins can affect tumor growth and metastasis through regulation of transcription of tumor-associated proteins. In that context, the polypyrimidine tract binding protein 1 (PTBP1) has been associated with head and neck squamous cell carcinoma (HNSCC). We aimed to further investigate the role of PTBP1 in head and neck cancers.

Methods: Using siRNA-mediated knockdown we investigated the role of PTBP1 in HNSCC cell lines, via proliferation assays, qPCR and Western blot analyses. Furthermore, we characterized the effect of si*PTBP1* using a proteomics approach. In addition, we assessed PTBP1 protein expression in tumor and healthy tissue using immunohistochemical approaches.

Results: PTBP1 expression was enhanced in head and neck tumors. A knockdown of PTBP1 in HNSCC cell line cells reduced proliferation, paralleled by an altered mRNA-profile and enhanced death receptor 5 (DR5) protein expression. In line with that, caspase-8 levels were also increased. Finally, we validated our findings from the cell culture, and found decreased DR5 levels in tumors with increased PTBP1 levels.

Conclusion: Our data indicate that targeting PTBP1 expression and thereby affecting DR5 expression might be a potential personalized therapeutic approach for HNSCC treatment in the future, especially for therapy-resistant HNSCC patients.

Poster Session

P5
Modulation of tumor metabolism to improve antitumor immune response in HNSCC

I. Ugele[1], S. Decking[1], K. Dettmer[2], L. Symeou[1], I. Michaelides[1], M. Rink[1], J. Künzel[1], P. Oefner[2], C. Bohr[1], K. Renner[1]

[1]University Hospital Regensburg, Klinik und Poliklinik für Hals-Nasen-Ohren-Heilkunde, Regensburg, Germany
[2]University Regensburg, Institute for functional genomics, Regensburg, Germany

Objectives: HNSCC has a poor prognosis and checkpoint inhibitors demonstrate limited efficacy. Survival in general and response to immunotherapy depend in particular on intratumoral T cell function, whose activity is impaired by the metabolic microenvironment. Given the observed decrease in glutamine levels in HNSCC, effects of pharmacological manipulation of glutamine metabolism were investigated.

Methods: Immune infiltrate, function and ROS levels were analyzed by flow cytometry, Interferon-g was quantified by ELISA. Metabolites were analyzed by mass spectrometry. Pharmacological manipulations were performed ex vivo in fresh tumor fragments and a tumor spheroid immune cell co-culture model.

Results: Overall survival was dependent on the portion of CD4+CD45+ T cells, which in turn correlated positively with intratumoral glutamine levels. Furthermore, glutamine restriction inhibited T cell function. Administration of BPTES, a selective glutaminase inhibitor, significantly increased Interferon-g levels in fresh tumor specimens. Notably, BPTES had little effect on extracellular glutamine concentrations, however, induced ROS levels and reduced viability in tumor cells but not in T cells.

Conclusion: It can be concluded, that treatment with BPTES impairs tumor cell redox status, most likely by interfering with the synthesis of the antioxidant glutathione. This limits tumor cell viability which may stimulate intratumoral T cell activity. In future, BPTES will be combined with immune checkpoint inhibitors.

Poster Session

P6
Platelet-mediated induction of epithelial-mesenchymal transition in head and neck squamous cell carcinoma

C. C. Hoch[1], Y. Han[1], K. Hachani[1], G. Multhoff[2], A. Bashiri Dezfouli[1,2], B. Wollenberg[1]

[1]TUM School of Medicine and Health, Technical University of Munich (TUM), Department of Otolaryngology, Head and Neck Surgery, Munich, Germany
[2]TUM School of Medicine and Health, Technical University of Munich (TUM), Central Institute for Translational Cancer Research, Department of Radiation Oncology, Munich, Germany

Question: In primary carcinomas, epithelial-mesenchymal transition (EMT) is a key process in cancer cell motility and metastasis, driven by stromal-derived growth factors, cytokines, and extracellular vesicles (EVs). As tumor cells enter the bloodstream, they may encounter new signaling modulators, which can augment their metastatic capacity. Platelets (PLTs), rich in a variety of growth factors, cytokines, and EVs, may therefore contribute to the EMT process, by promoting metastasis. This study aimed to ascertain whether PLTs can induce EMT in head and neck squamous cell carcinoma (HNSCC) cells.

Methods: HNSCC cell line (SAS cells) was co-cultured with either activated or non-activated PLTs from healthy donors, as well as tumor-educated PLTs (TEPs). Flow cytometry was employed to assess the expression of PLT surface activation markers (CD107a, CD62p, CD63, and CD40L) following co-incubation with SAS cells. The expression of EMT surface markers (CD324, CD326, CD104, CD44, and vimentin) was evaluated on SAS cells at three time points (3, 6, and 9 days)-before and after co-culturing with PLTs and TEPs.

Results: Initial characterization of SAS cells demonstrated an epithelial phenotype, with robust expression of CD324, CD326, and CD104. Following a three-day co-culture with both PLTs and TEPs, a significant decline in epithelial surface marker expression (CD324, CD326, CD104) was observed. By day 6, this effect persisted, along with an increase in mesenchymal surface marker expression (CD44, vimentin). Co-culturing of HNSCC cells with PLTs and TEPs resulted in a notable shift from an epithelial to a mesenchymal phenotype, suggesting a potential role for PLTs and TEPs in promoting metastasis. A comparative analysis over time revealed distinct EMT induction patterns between PLTs and TEPs.

Conclusions: Our study provides new insights into the interaction between platelets and tumor cells suggesting that platelets could serve as therapeutic targets to inhibit EMT and reduce metastasis in HNSCC patients.

Poster Session

P7
Innovative gold-based nanocarrier strategy to enhance therapy in head and neck squamous cell carcinoma

F. van Petten Azevedo[1], C. Abbehausen[2], E. de Paula[3], I. Diniz[1], G. Gerônimo[3], G. J. Lourenço[1], C. Lima[1]

[1]University of Campinas - UNICAMP, School of Medical Sciences, Campinas, Brazil
[2]University of Campinas - UNICAMP, Institute of Chemistry, Campinas, Brazil
[3]University of Campinas - UNICAMP, Institute of Biology, Campinas, Brazil

Objective: To investigate the therapeutic potential of a novel gold-based compound integrated within a lipid nanocarrier system to address the high recurrence, invasiveness, and treatment resistance in head and neck squamous cell carcinoma (HNSCC). The study aims to evaluate the compound"s efficacy in reducing cell viability, proliferation, adhesion, and migration in HNSCC models, and to explore preliminary mechanisms of action.

Methods: HNSCC cell lines were treated with the gold-based compound delivered through a lipid-based nanocarrier. Cell viability and proliferation were assessed using standard cytotoxicity assays. Cell adhesion and migration were evaluated to determine anti-metastatic effects. Preliminary mechanistic studies focused on oxidative stress pathways and key signaling processes related to cancer cell survival. Data analysis was performed using appropriate statistical methods to ensure validity and reliability.

Results: The gold compound delivered via the lipid nanocarrier significantly reduced cell viability and proliferation in HNSCC cell lines, demonstrating strong anti-metastatic effects through decreased cell adhesion and migration. Preliminary findings suggest that the compound may modulate oxidative stress and disrupt critical signaling pathways essential for tumor cell survival, though further studies are required to confirm these mechanisms.

Conclusion: This study presents a promising approach for targeted HNSCC therapy using a gold-based nanocarrier system, with potential to overcome current limitations in treatment by enhancing compound bioavailability and minimizing toxicity. Further in vivo studies are planned to validate efficacy, optimize the delivery system, and assess safety profiles, with the goal of advancing this strategy towards clinical applications for improved therapeutic outcomes in HNSCC.

Keywords: HNSCC, gold-based therapy, lipid nanocarrier, cell viability, metastasis, oxidative stress, targeted therapy

Poster Session

P8
Anti-proliferative activity of a palladium(II) complex over squamous cell carcinoma of tongue

T. Z. Candido[1], J. E. d. Carvalho[2], A. L. T. Ruiz[2], P. P. Corbi[3], G. J. Lourenço[1], C. Lima[1]

[1]University of Campinas - UNICAMP, Faculty of Medical Sciences, Campinas, Brazil
[2]University of Campinas - UNICAMP, Faculty of Pharmaceutical Sciences, Campinas, Brazil
[3]University of Campinas - UNICAMP, Institute of Chemistry, Campinas, Brazil

Introduction: Squamous cell carcinoma of head and neck is considered (SCCHN) is one of the most prevalent tumors in word. Treatments include surgical resection, radiotherapy, and chemotherapy in the cases of Cisplatin (CP) has been used for treatment of patients with advanced SSCHN. Nevertheless, patients treated with CP are subjected to adverse effects as nephrotoxicity, and the search for new chemotherapeutic agents with reduced side effects is required. Padeliporfin (Tookad®Soluble) was the first palladium(II) complex used in vascular targeted photochemotherapy for treatment of patients with prostate cancer, which also confirms the potential of use of this metal in the synthesis of new chemotherapeutic agents. Our research group has dedicated efforts in the search of novel palladium complexes for treatment of SCC. One of the silver complexes with the anti-inflammatory drug nimesulide recently prepared in our group demonstrated *in vitro* and *in vivo* activity over SCC cells.

Objectives: This study aimed to present the in vitro anti-proliferative activities over SCC of a water-soluble palladium(II) complex containing a cysteine derivative as a chelating ligand.

Materials and Methods: SCC of tongue (SCC4 and SCC15) and a non-tumoral cell line (HaCat, immortalized keratinocyte) were used in this study. The cells were cultivated following methodology previously described in the literature.

Results: The palladium(II) complex inhibited proliferation of SCC15 cells with a GI50 (concentration of a drug that reduces cell growth by 50%) of 40.28 µg mL 1 but low selectivity was observed when compared to HaCat cells (GI50: 28.33 µg mL 1). On the other hand, the complex did not inhibit SCC4 cell proliferation (GI50 > 250 µg mL 1).

Conclusion: The palladium(II) complex seems to be indicated for treatment of SCC of tongue, but further studies are envisaged to understand the selectivity of the complex over the considered SCC lines and propose its possible mechanism of action.

Poster Session

P9
In vitro conditioning of NK cells through exposure to HNSCC-derived exosomes

A. Bashiri Dezfouli[1,2], K. Hachani[1], C. C. Hoch[1], M. Yazdi[3], G. Multhoff[2], B. Wollenberg[1]

[1]TUM School of Medicine and Health, Technical University of Munich (TUM), Department of Otolaryngology, Head and Neck Surgery, Munich, Germany
[2]Central Institute for Translational Cancer Research, Technical University of Munich (TranslaTUM), Department of Radiation Oncology, Klinikum rechts der Isar, Munich, Germany
[3]Ludwig Maximilian University of Munich, Pharmaceutical Biotechnology, Department of Pharmacy, Munich, Germany

Question: Malignancies such as head and neck squamous cell carcinoma (HNSCC) engage in a complex interaction with the immune system, employing mechanisms to escape from immune-mediated clearance. Small extracellular vesicles (e.g. exosomes) released by tumor cells play key roles in tumor-host communication and modulating the immune system, supporting the tumor progression. Our study focuses on tumor-derived exosomes (TEX) from HNSCC cell lines (UD5, Cal27, and SAS cells), aiming to examine their physical characteristics as well as their impact on the surface receptor profile and activity of natural killer (NK) cells.

Methods: TEX were isolated from cell culture supernatants using size-exclusion chromatography, with size and morphology characterized by dynamic light scattering and transmission electron microscopy. Surface markers, including tetraspanins and heat shock protein 70 (Hsp70), were acquired by flow cytometry. Hsp70-positive TEX binding to the cmHsp70.1 monoclonal antibody (mAb) was assessed via microscale thermophoresis. NK cell receptor expression profile was evaluated upon TEX co-incubation, and killing activity of TEX-treated NK cells against HNSCCs was measured in a 4-h co-culture.

Results: TEX derived from UD5, Cal27, and SAS cells displayed uniform size distributions and expressed exosomal markers, including CD9, CD63, CD81, and the tumor-specific antigen, Hsp70. All TEX sources showed high binding affinity to the cmHsp70.1 mAb. Notably, treatment with these TEX downregulated NK cell-activating receptors, along with an inconsistent increase in inhibitory receptors such as NKG2A and PD-L1. Additionally, NK cell cytotoxic activity against HNSCCs was reduced following TEX exposure.

Conclusions: Our findings suggest that TEX may facilitate tumor immune evasion by reducing NK cell activation and cytotoxic potential, indicating their potential as valuable biomarkers and therapeutic targets to counteract tumor-induced immune suppression.

Poster Session

P10
Intratumoral T-cell abundance and antigen specific immune responses in HPV positive and negative head and neck cancer

H. Eckel[1], J. P. Klußmann[1], H. A. Schlößer[2]

[1]University of Cologne, Abteilung für Hals,- Nasen,- Ohrenheilkunde, Kopf- & Halschirurgie, Cologne, Germany
[2]University of Cologne, Klinik und Poliklinik für Allgemein-, Viszeral-, Tumor- und Transplantationschirurgie, Cologne, Germany

Background: Intratumoral T-cell abundance is an established predictor of improved survival in and neck squamous cell carcinoma (HNSCC), likely due to improved CD8 T cell mediated tumor killing. Antigen-specific immune response is a hallmark of cancer immunotherapy. Potential antigens in HNSCC include tumor-associated antigens (TAAs), mutation-associated neoantigens (MANAs), and viral proteins, including high-risk human papillomavirus (HPV) proteins. Studies combining multiple antigens for cellular therapy renewed interest in TAAs. While intratumoral expression of antigens is frequent, immunotherapy does not induce durable tumor regression in most HNSCC patients.

Methods: We analyzed intratumoral T-cell abundance in a large retrospective cohort of HNSCC patients using an adapted immune score. RNA expression of TAAs and genes associated with antigen presentation were assessed by 3" RNA Sequencing of treatment-naïve HPV-positive and HPV-negative HNSCC tumor samples and matching healthy mucosa. Endogenous T cell and humoral responses against viral proteins and TAAs were determined by FluoroSpot and protein-bound bead assays. Expression of components of the HLA class I antigen presentation pathway was analyzed in a large cohort of HPV-positive and HPV-negative HNSCC patients and correlated to intratumoral immune cell abundance.

Results/Conclusion: Increased intratumoral T-cell infiltration was associated with improved survival and positive HPV status. Endogenous antigen-specific T-cell responses against viral antigens and TAAs were frequently detectable, and nuanced differences in relation to p16 and HPV DNA status were identified. Humoral responses directed at the same antigens were partially overlapping with T-cell responses. New results will be presented in the context of previously published literature regarding antigen-specific immune response and antigen expression in HNSCC.

Poster Session

P11
Two distinctive molecular subgroups of patients with specific expression phenotypes determine the majority of recurrences in advanced laryngeal carcinoma

T. Popov[1], G. Stancheva[2], S. Kyurkchiyan[2], V. Petrova[2], R. Kaneva[2]

[1]Medical University - Sofia, Department of ENT, Sofia, Bulgaria
[2]Medical University - Sofia, Molecular Medicine Center, Sofia, Bulgaria

Question: Delineate independent molecular subgroups of patients that strongly correlate with recurrence and survival outcomes with focus on tumor heteroguenity.

Methods: 60 patients with advanced laryngeal carcinoma were enrolled in the current study. Samples from each patient: tumor site –surface, tumor site-depth, histologically healthy peritumor mucosa and paired normal laryngeal mucosa distant to the tumor as a control sample. mRNA levels of the major proangiogenic molecules (HIF-1α, HIF-2α, HIF-3α VEGF-A, VEGFR1, VEGFR2, ETS-1) were measured and data from a global microarray microRNA profile on the same cohort, validated with RT-PCR, was integrated in the analysis.

Results: VEGF-A displayed very strong association with recurrence in advanced laryngeal carcinoma **exclusively and only** when measured in tumor depth (Log-rank, p=0.0001, $\chi 2 = 14.8$). In stark contrast, it"s expression levels in tumor surface have no prognostic value (Log rank, p=0.170). A specific subgroup of patients with upregulated mRNA levels of VEGF-A in tumor depth (RQ>2) and normal/downregulated mRNA levels of HIF-1α (RQ<2) exhibit extraordinarily high levels of recurrence rate (64% recurrence rate vs a 28.5% for the rest of the cohort, p=0.028). Another distinctive subgroup of patients, which is independent from the latter, with significantly worse prognosis exhibits higher levels of co-expression of miR-93-5p, miR-144-3p, and miR-210-3p ($\chi 2(2)=4.68$, log-rank p=0.03; $\chi 2(2)=4.53$, log-rank p=0.03, $\chi 2(2)=4.53$, log-rank p=0.03, respectively). Both subgroups comprise remarkably 76% of all recurrences in the studied cohort.

Conclusion: We delineate two distinctive subgroups based on molecular profiles which comprise the majority of recurrences (76%) in advanced laryngeal carcinoma. We demonstrate that VEGF-A heterogeneity in laryngeal carcinoma is evident and clinically important in terms of prognostic value – only tumor depth expression levels provide consistent prognostic value.

Fig. 1

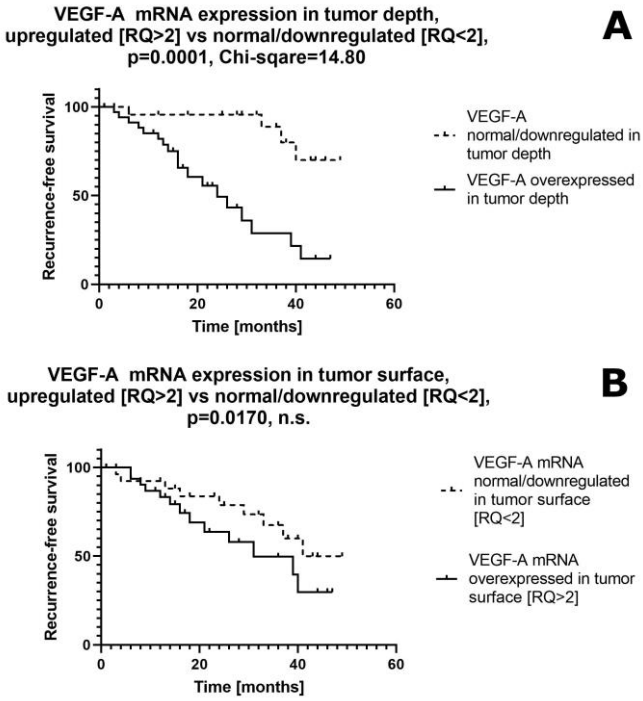

VEGF-A mRNA expression in tumor depth, upregulated [RQ>2] vs normal/downregulated [RQ<2], p=0.0001, Chi-sqare=14.80
A
Recurrence-free survival
100
50
0
0
20
40
60
Time [months]
VEGF-A normal/downregulated in tumor depth
VEGF-A overexpressed in tumor depth
VEGF-A mRNA expression in tumor surface, upregulated [RQ>2] vs normal/downregulated [RQ<2], p=0.0170, n.s.
B
Recurrence-free survival
100
50
0
0
20
40
60
Time [months]
VEGF-A mRNA normal/downregulated in tumor surface [RQ<2]
VEGF-A mRNA overexpressed in tumor surface [RQ>2]

Fig. 2

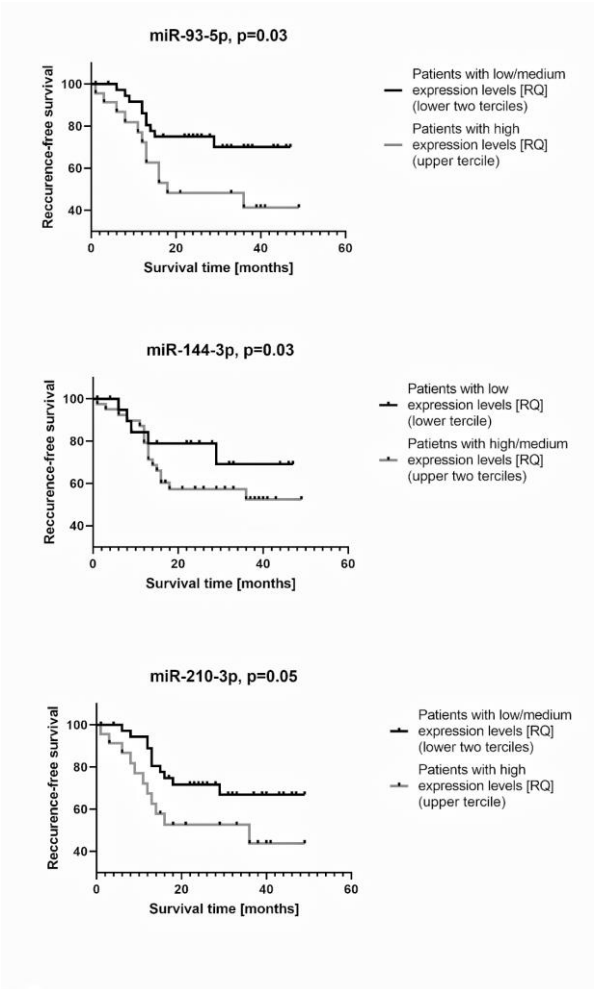
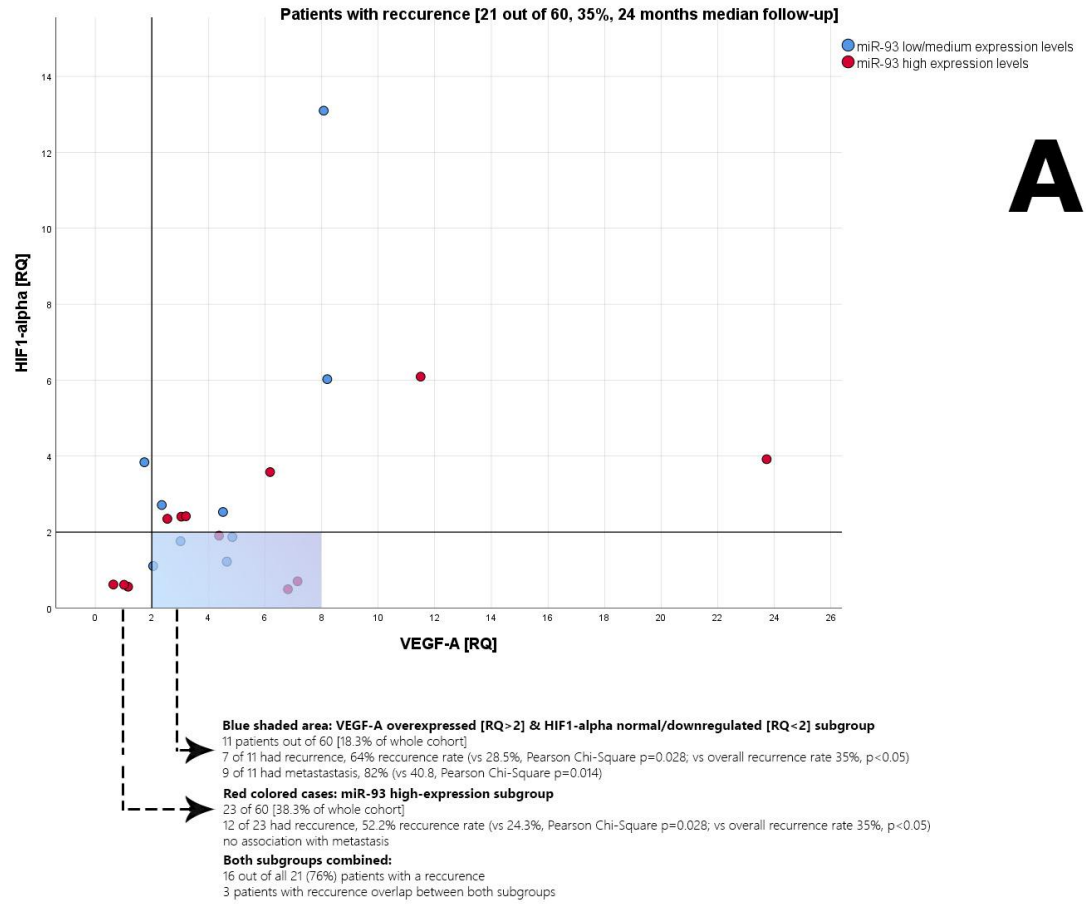
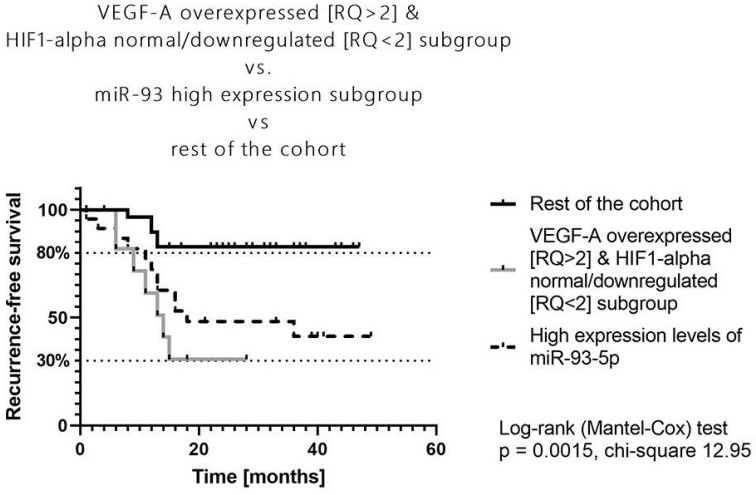

miR-93-5p, p=0.03
Reccurence-free survival
100
80
60
40
0
20
40
60
Survival time [months]
Patients with low/medium expression levels [RQ] (lower two terciles)
Patients with high expression levels [RQ] (upper tercile)
miR-144-3p, p=0.03
Reccurence-free survival
100
80
60
40
0
20
40
60
Survival time [months]
Patients with low expression levels [RQ] (lower tercile)
Patietns with high/medium expression levels [RQ] (upper two terciles)
miR-210-3p, p=0.05
Reccurence-free survival
100
80
60
40
0
20
40
60
Survival time [months]
Patients with low/medium expression levels [RQ] (lower two terciles)
Patients with high expression levels [RQ] (upper tercile)

Fig. 3


Patients with reccurence [21 out of 60, 35%, 24 months median follow-up]
miR-93 low/medium expression levels
miR-93 high expression levels
A
HIF1-alpha [RQ]
VEGF-A [RQ]
Blue shaded area: VEGF-A overexpressed [RQ>2] & HIF1-alpha normal/downregulated [RQ<2] subgroup
11 patients out of 60 [18.3% of whole cohort]
7 of 11 had recurrence, 64% reccurence rate (vs 28.5%, Pearson Chi-Square p=0.028; vs overall recurrence rate 35%, p<0.05)
9 of 11 had metastastasis, 82% (vs 40.8, Pearson Chi-Square p=0.014)
Red colored cases: miR-93 high-expression subgroup
23 of 60 [38.3% of whole cohort]
12 of 23 had reccurence, 52.2% reccurence rate (vs 24.3%, Pearson Chi-Square p=0.028; vs overall recurrence rate 35%, p<0.05)
no association with metastasis
Both subgroups combined:
16 out of all 21 (76%) patients with a reccurence
3 patients with reccurence overlap between both subgroups


VEGF-A overexpressed [RQ>2] &
HIF1-alpha normal/downregulated [RQ<2] subgroup
vs.
miR-93 high expression subgroup
vs
rest of the cohort
B
Recurrence-free survival
Time [months]
Rest of the cohort
VEGF-A overexpressed [RQ>2] & HIF1-alpha normal/downregulated [RQ<2] subgroup
High expression levels of miR-93-5p
Log-rank (Mantel-Cox) test
p = 0.0015, chi-square 12.95

Poster Session

P12
Neural progenitors driving neurogenesis in head and neck cancer and its role in tumor development

S. Liang[1], K. Khorani[1], R. Han[1], Y. Xie[1], J. Hess[1]

[1]University of Heidelberg, Heidelberg, Germany

Background: Nerve-cancer crosstalk has emerged as a new research topic in recent years, and several studies have demonstrated a critical role of neural components in the tumor microenvironment (TME) during carcinogenesis by interacting with tumor and immune cells. A better understanding of neurogenesis in the TME of head and neck cancer may provide new nerve-targeted treatment options with the ultimate goal of improving patient prognosis.

Aims: To investigate the underlying principles of neurogenesis in head and neck cancer and to explore the cellular and molecular mechanisms of tumor innervation and tumor progression.

Methods: A neural stem/progenitor cell (NSC/NPC) related gene set was extracted using PanglaoDB and applied to RNA-seq data from TCGA-HNSC (n=520). After propensity score matching using clinical baseline information, an integrative analysis of multi-omics data from 282 patients was performed to identify differences in genetic and epigenetic landscapes as well as immune phenotype. Finally, the association of the intra-tumoral neurogenesis gene profile with prognosis was investigated.

Results: Based on the NSC/NPC-related 58-gene set, tumors were divided into group A (high NSC/NPC-related genes) and group B (low NSC/NPC-related genes) with significant differences in the gene expression of characteristic markers, such as doublecortin (DCX), NEUROD1, SOX3, and POU3F4. Tumors in group A were associated with molecular features related to neuro-inflammatory response, IFN-α and IFN-γ signaling, but showed lower expression of gene sets related to neuronal development, differentiation and function. Analysis of tumor-infiltrating immune cells revealed that the TME of tumors in group A had a higher proportion of CD8+ T cells, activated NK cells, M1 and M2 macrophages, while the TME of tumors in group B had a higher proportion of plasma cells, Tregs and M0 macrophages.

Conclusion: The study confirms that gene expression related to early neurogenesis is a common feature in head and neck cancer and enables the identification of molecular subgroups with distinct pathway activities and immune phenotypes.

Poster Session

P13
miR-4421 as a possible modulator of MAPK/Akt pathway through ERP29 in pharyngeal cancer

J. Carron[1], C. Lima[1], G. J. Lourenço[1]

[1]University of Campinas - UNICAMP, Campinas, Brazil

Question: Our previous study identified the binding affinity between the microRNA miR-4421 and *ERP29* gene, which encodes a chaperone protein. This interaction leads to *ERP29* silencing and may play a role in influencing pharyngeal cancer (PC) risk and progression. However, the precise mechanism underlying this process is still unknown, especially the influence of known signaling pathways, as MAPK and Akt. This study aims to explore the role of miR-4421 and *ERP29* in PC survival and progression.

Methods: We assessed *ERP29* and miR-4421 prognostic value with Kaplan–Meier Plotter. We identified and validated genes modulated by *ERP29* in FaDu cisplatin-sensitive and resistant (FaDu-R) cells. We tested if miR-4421 inhibitor could reverse *ERP29* silencing effect, with gene expression analyzed by qPCR. Statistical analysis was performed by *t*-test.

Results: Lower *ERP29* (p= 0.03) and higher miR-4421 (p< 0.01) expression was linked to poor survival. In FaDu cells, *ERP29* silencing increased *MAPK1* (FC: 2.4, p= 0.03), *AKT1* (FC: 17.5, p< 0.01), and *JUN* (FC: 29.0, p= 0.01) expression when compared to cells expressing *ERP29*. In contrast, the transfection of miR-4421 inhibitor revert those effects, decreasing the expression of *MAPK1* (FC: 0.6, p= 0.03), *AKT1* (FC: 0.1, p= 0.02), and *JUN* (FC: 0.1, p= 0.02) compared to the negative control. In FaDu-R cells, *ERP29* silencing increased *SOS1* (FC: 2.2, p< 0.01), *MAPK1* (FC: 2.1, p< 0.01), and *AKT1* (FC: 2.2, p= 0.04) expression when compared to cells expressing *ERP29*. Conversely, miR-4421 inhibitor decreased the expression of *SOS1* (FC: 0.2, p= 0.03), *MAPK1* (FC: 0.4, p= 0.01), and *AKT1* (FC: 0.2, p= 0.04) compared to the negative control.

Conclusions: Loss of *ERP29* expression may impact MAPK and Akt pathways, contributing to PC patients" poor survival. However, these effects could be reversed by inhibiting the binding of miR-4421 to *ERP29*. Our study could assist in the development of targeted therapy for PC patients based on ensuring *ERP29* expression.

Poster Session

P14
Effect of ERP29 silencing on PI3K/AKT pathway gene expression in cisplatin-sensitive and resistant pharyngeal cancer cells

J. Carron[1], C. Lima[1], G. J. Lourenço[1]

[1]School of Medical Sciences of University of Campinas, Laboratory of Cancer Genetics, Campinas, Brazil

Objective: Silencing the chaperone ERp29 has been associated with increased progression of pharyngeal tumor cells, suggesting that ERp29 may inhibit aggressive tumor behavior. However, the mechanisms underlying this effect remain unclear. This study aimed to evaluate the expression patterns of genes in the PI3K/AKT pathway in FaDu, FaDu cisplatin-treated (FaDu-CDDP), and FaDu cisplatin-resistant (FaDu-R) cell lines following ERp29 suppression.

Methods: *ERP29* was silenced using small interfering RNA. The TaqMan Array Human Molecular Mechanisms of Cancer was used to identify potential genes in the PI3K/AKT pathway modulated by *ERP29*, with results validated by qPCR and analyzed using a *t*-test.

Results: *SRC* expression was higher in FaDu-CDDP cells compared to FaDu (fold-change (FC): 3.4, p= 0.02) and FaDu-R (FC: 4.6, p< 0.001), but in cells with *ERP29* silencing, *SRC* levels were similar across cell lines. *AKT1* was more expressed in both FaDu (FC: 4.2, p= 0,03) and FaDu-CDDP (FC: 3.9, p= 0.04) cells compared to FaDu-R. However, *AKT1* expression was increased in FaDu cells than in FaDu-CDDP (FC: 1.7, p= 0.04) in silenced *ERP29* cells. No significant differences in *ITGAV* expression were observed among the cell lines. Following silencing, *ITGAV* expression was higher in FaDu (FC: 3.3, p= 0.02) and in FaDu-R (FC: 2.3, p= 0.01) cells compared to FaDu-CDDP. *JUN* expression was higher in FaDu-CDDP compared to FaDu-R (FC: 2.6, p= 0.04), but in cells with *ERP29* silencing, *JUN* expression was increased in FaDu cells than in the others (FC: 4.5, p= 0.03 and FC: 3.0, p= 0.03). *MDM2* expression was lower in FaDu than others two cell lines (FC: 0.3, p= 0.002 and FC: 0.2, p= 0.01). In *ERP29* silencing cells, *MDM2* expression was higher in FaDu cells compared to FaDu-CDDP (FC: 2.0, p= 0.02).

Conclusion: These findings highlight the role of *ERP29* in modulating key genes in the PI3K/AKT pathway, potentially influencing tumor cell behavior in pharyngeal cancer.

Poster Session

P15
Adipocytes enhance tongue cancer progression: Possible involvement of adipokine IL-6 and extracellular vesicles

K. Juurikka[1,2], J. Peltonen[3], R. Tiikkaja[4,2], L. Ketomäki[4,2], T. Sandvik[4,2], T. Kaakkuriniemi[4,2], S. Kokkonen[4,2], J. Heikkinen[2,5], S. Palosaari[2,5], J. Väisänen[2,6], J. Tikanto[2,6], P. Koivunen[2,6], A. Al-Samadi[3,7], M. Risteli[4,2], T. Salo[3,4,2,8], P. Åström[1,2,9]

[1]University of Oulu, Research Unit of Biomedicine and Internal Medicine, Oulu, Finland
[2]Medical Research Center, Oulu, Finland
[3]University of Helsinki, Department of Oral and Maxillofacial Diseases, Helsinki, Finland
[4]University of Oulu, Research Unit of Population Heath, Oulu, Finland
[5]University of Oulu, Research Unit of Translational Medicine, Oulu, Finland
[6]Oulu University Hospital, Oulu, Finland
[7]University of Eastern Finland, Institute of Dentistry, Kuopio, Finland
[8]Helsinki University Central Hospital, Department of Pathology, Helsinki, Finland
[9]Biocenter Oulu, Oulu, Finland

Objective: Obesity is associated with increased incidence in many cancers1,2, yet the association with oral cancer risk is partly controversial3,4. The mechanistic role of adipocytes is largely unknown. We studied the prognostic value and effect of adipose tissue in oral tongue squamous cell carcinoma (OTSCC) progression.

Methods: Amount and location of adipocytes and inflammatory cells was evaluated from OTSCC tumor samples. OTSCC cells were co-cultured with adipocytes differentiated from mesenchymal stem cells or adipose tissue from oral cancer patients. Cell proliferation, viability, motility, secretion of adipokines and epithelial-mesenchymal marker levels were measured with functional assays and molecular biology methods. Lastly extracellular vesicles (EVs) were extracted from differentiated adipocytes and their content and effect on OTSCC motility were studied.

Results: Adipocyte amount in OTSCC samples positively correlated with tumor size. High inflammatory cell count predicted better overall survival. Patient-derived adipose tissue and differentiated adipocytes induced proliferation of OTSCC cells *in vitro*. Adipocytes increased the migration of cancer cells from both primary and metastatic sites without direct cell-cell contact. Differentiated adipocytes and adipose tissue in co-culture with OTSCC cells produced various adipokines including interleukin 6 (IL-6), and inhibition of IL-6 signaling markedly reduced cell migration. Lastly, the adipocyte-derived EVs contained IL-6 and induced OTSCC cell invasion.

Conclusion: Adipocytes increase the proliferation of cancer cells and enhance their motility independent of direct cell contact. The protumorigenic effect of adipocytes is likely mediated by secreted cytokines, such as IL-6 and transported via EVs.

References:

1De Pergola & Silvestris (2013) J Obes 291546.

2Park et al. (2014) Nat Rev Endocrinol 10:455-465.

3Kreimer et al. (2006) Int J Cancer 118:2293-7.

4Iyengar et al. (2014) Cancer 120:983-91.

Poster Session

P16
Self-supervised analyses of head and neck cancer histologies reveal novel malignant growth pattern

M. Knebel[1], F. Ersan[2], J. Hense[3], C. Walz[1], K. R. Müller[3], S. Otto[2], F. Klauschen[1], P. Liokatis[2], A. Mock[1]

[1]Institute of Pathology LMU Munich, Munich, Germany
[2]Ludwig Maximilian University of Munich, Deparment of Oral and Maxillofacial Surgery and Facial Plastic Surgery, Munich, Germany
[3]Technische Universität Berlin, Machine Learning Group, Department of Electrical Engineering and Computer Science, Berlin, Germany

Question: This study aims to dissect the morphological spectrum of head and neck squamous cell carcinoma (HNSCC) in primary tumors and lymph node metastases and interrogate impact on progression-free and overall survival and associated biological characteristics.

Methods: The cohort consisted of 2987 diagnostic whole-slide images from 115 HNSCC cases with matched primary tumors and lymph node metastases archived at the Institute of Pathology, LMU Munich. The computational pathology workflow included segmentation, tile extraction and stain normalization, followed by foundation model-based (CTransPath) feature extraction. The resulting feature landscape was clustered by Leiden algorithm. The histological properties and their impact on progression-free and overall survival were assessed. Whole-slide images of the TCGA cohort (472 slides from 450 cases) were used as a validation cohort and the underlying biological properties of morphological clusters could be investigated by comparative functional genomics in the RNA-seq data.

Results: Fondation model-based feature extraction revealed a morphological landscape consisting of 31 clusters. One cluster was significantly associated with a poorer progression-free survival. This association could be confirmed in the TCGA validation cohort. Integration of morphological clusters with RNA-seq data in the TCGA data revealed upregulation of genes linked to stem cell-like functions within this pattern, suggesting a higher plasticity as a putative reason for the more aggressive tumor biology.

Conclusion: This study demonstrates the potential of self-supervised learning to identify clinically meaningful growth patterns in HNSCC. We are currently aiming for a deeper biological characterization by spatial transcriptomics.

Poster Session

P17
Reevaluation of the immunotherapy Bayesian network model for head and neck cancer

M. Stoehr[1], A. Dietz[1], J. Gaebel[2]

[1]University Hospital Leipzig, Department of Otorhinolaryngology, Head and Neck Surgery, Leipzig, Germany
[2]University Leipzig, Innovation Center Computer Assisted Surgery, Leipzig, Germany

Objective: Oncological decision-making processes are becoming increasingly complex with advances in diagnostics and more individual therapy options. In the case of head and neck tumors (HNC), this requires new information processing techniques and suitable models to support the decision-making process in the head and neck tumor board (HNTB) and molecular tumor board (MTB). For this purpose, a molecular pathological model was developed on the basis of the digital patient model for HNC.

Methods: As a submodel of the HNC model, the head and neck immunotherapy model (HNITM) was developed as a Bayesian network utilizing the software GeNIe 2.0 and evaluated with 25 patient records successfully. The HNITM was created on the basis of recent guidelines and studies. The graph structure was optimized and newly established therapy methods were integrated.

Results: The HNITM can evaluate patient cases regarding the potential treatment with the immune checkpoint inhibitors pembrolizumab or nivolumab. Initially, the HNITM showed an accuracy of 84%. After reevaluation with further 70 HNC cases, the HNITM could be confirmed and revealed improved prediction of the cases according to the indication and the current guidelines.

Conclusion: Personalized medicine and targeted therapy are of increasing importance in oncological therapy and require structured and comprehensive support for information management and decision-making. Taking into account the current guidelines and studies, the model can estimate suitable treatment options by reliably calculating probabilities and thus provide support for immunotherapy in HNC. The model is to be expanded through optimization in order to optimize the therapy decision-making processes in HNC patients.

Supported by:

Federal Ministry of Education and Research

Poster Session

P18
Spatial distribution of leukocyte subsets affects development of head and neck squamous cell carcinoma

C. Netzer[1,2], V. von Arps Aubert[2], I. Mačinković[3], J. von der Grün[4,5], S. Küffer[6], P. Ströbel[6], A. von Knethen[7], A. Weigert[3], D. Beutner[2]

[1]Heidelberg University Hospital, Department of Otorhinolaryngology, Head and Neck Surgery, Heidelberg, Germany
[2]University Medical Centre Göttingen, Department of Otorhinolaryngology, Head and Neck Surgery, Göttingen, Germany
[3]Faculty of Medicine, Goethe-University Frankfurt, Institute of Biochemistry I, Frankfurt, Germany
[4]University Hospital Zurich and University of Zurich, Department of Radiation Oncology, Zurich, Switzerland
[5]University Hospital Frankfurt, Department of Radiotherapy and Oncology, Frankfurt, Germany
[6]University Medical Centre Göttingen, Institute of Pathology, Göttingen, Germany
[7]University Hospital Frankfurt, Goethe-University Frankfurt, Department of Anesthesiology, Intensive Care Medicine and Pain Therapy, Frankfurt, Germany

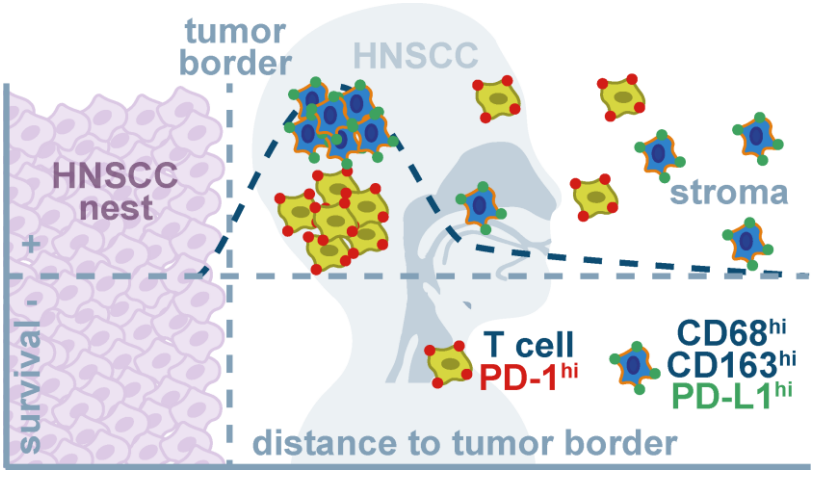
Background: Development and metastasis of head and neck squamous cell carcinoma (HNSCC) involve complex interactions between tumor cells and the microenvironment. Spatial cell arrangement significantly influences these interactions.

Methods: The spatial distribution of leukocyte subsets in HNSCC was analyzed by multiplex immunohistochemistry (IHC) and correlated with clinical outcome data. In addition, leukocyte subsets were classified using single-cell mRNA datasets and flow cytometry (FC).

Results: IHC revealed characteristic leukocyte distribution patterns based on CD68 and CD163 expression. CD68hiCD163lo and CD68hiCD163hi cells accumulated close to tumor foci, whereas CD68loCD163hi cells were more evenly distributed in the tumor stroma. PD-L1hi and PD-1hi cells accumulated predominantly near tumor foci. High PD-L1hi CD68hiCD163hi or PD-1hi T cell density near tumor sites correlated with improved survival. FC and RNA analysis showed a high heterogeneity of CD68/CD163 subsets. CD68hiCD163lo and CD68hiCD163hi cells were primarily macrophages (MΦ), while CD68loCD163hi cells were mainly formed by dendritic cells (DCs). Differentiation by CD64, CD80, CD163 and CD206 indicated a different polarization within macrophages (MΦ). MΦ expressed predominantly CD206 and little CD80. The opposite was observed in DCs.

Conclusion: Spatial distribution, cell interactions and surface protein expression indicate different roles of CD68/CD163 subsets in the HNSCC microenvironment. Whether PD-L1/PD-1 interactions positively or negatively affect survival depends on the type and localization of interacting cells. These results emphasize the relationship between the spatial distribution of leukocytes and the clinical presentation of HNSCC.

Fig. 1



Poster Session

P19
Mapping molecular subtypes in HNSCC: From bulk RNA to spatial data

S. M. Khan[1]

[1]German Cancer Research Center (DKFZ), Heidelberg, Germany

Question: Head and neck squamous cell carcinomas (HNSCC) exhibit significant heterogeneity, complicating prognosis and treatment strategies. The molecular subtyping by Walter et al. classifies HNSCC into four distinct subtypes based on bulk RNA data.[1] However, conflicting results suggest tumors may contain hybrid subtypes.[2] This study analyzes these subtypes using spatial transcriptomics to compare with bulk RNA results and assess the presence of hybrid subtypes within individual tumors.

Methods: We analyzed bulk transcriptome data from 13 untreated HNSCC xenograft models for alignment with the four molecular subtypes. Only two models consistently matched a specific subtype, while others showed diversity. We are investigating spatial transcriptomic data from 7 of these models to generate spatial subtype patterns by mapping distributions within the tumor microenvironment and comparing them with bulk RNA data.

Results: Preliminary findings indicate that HNSCC tumors may comprise multiple subtypes rather than a single one. Spatial transcriptomic analysis reveals intratumoral heterogeneity, suggesting hybrid subtypes. This spatial diversity correlates with variability observed in bulk RNA data across different models.

Conclusions: Our study suggests that the ratio of different subtypes within a tumor enhances the molecular understanding of HNSCC behavior. By integrating spatial transcriptomics with microenvironment features, radiomic signatures, and histopathological data, we aim to refine the molecular subtyping method. This refined classification could generate a translatable, subtype-specific molecular profile applicable to clinical HNSCC samples, ultimately improving prognosis and patient stratification.

References:

1. Walter V, et al. "Molecular subtypes in head and neck cancer..." *PLoS One*. 2013;8(2)
2. Keck MK, et al. "Integrative analysis of head and neck cancer..." *Clin Cancer Res*. 2015;21(4):870-81.

Poster Session

P20
Impact of the sex chromosome dosage on the tumor microenvironment in head and neck squamous cell carcinoma

C. Conde Lopez[1], D. Marripati[2], M. Elkabets[2], J. Hess[1], I. Kurth[1]

[1]German Cancer Research Center (DKFZ), E220, Heidelberg, Germany
[2]Ben-Gurion University of the Negev, Beer-Sheva, Israel

Head and neck squamous cell carcinoma (HNSCC) presents significant sex differences in incidence, prognosis, and treatment response, which are attributed to sex chromosome variations and hormone expression. This study exploresthese sex-specific differences, particularly focusing on the genetic foundations by examining sex chromosome dosage (XX, XY, XØ) and its effects on the tumor microenvironment (TME). Utilizing omics data from the TCGA-HNSC and single-cell datasets, our analysis applies advanced analytical methods such as the Seurat pipeline, scGate for cell annotation, and ProjectTILS for an in-depth examination of gene regulation, oncogenic pathways, and cell composition differences across different sex chromosome compositions. An important aspect of this analysis is the categorization of male patients into XY and XØ groups based on Y chromosome expression and comparison with the XX group to observe variations in TME composition, employing tools like Cellchat for detailed ligand-receptor interaction studies. The results show significant intratumoral heterogeneity and a correlation with HPV status, with distinct cell dynamics and signaling activities in the TME across XX, XY, and XØ groups. Particularly, the study highlights the role of fibroblasts, which exhibit high COX2/PTGS2 and AR expression, in modulating the TME. A balanced investigation into both the stromal and immune compartments and their communication with tumor cells becomes relevant, as their interplay may differ across sex chromosomal backgrounds, influencing the TME in unique ways. Our findings are expected to add valuable insights to the field of cancer research, providing new perspectives on how sex chromosomes influence cancer pathology and treatment strategies.

Poster Session

P21
Impact of combined SNVs on renal function in HNSCC patients undergoing Cisplatin-based treatment

E. F. D. Costa[1], A. M. C. Ferreira[1], M. Mazzali[1], C. D. Ramos[1], G. J. Lourenço[1], C. Lima[1]

[1]University of Campinas - UNICAMP, Faculty of Medical Sciences, Campinas, Brazil

Introduction: Head and neck squamous cell carcinoma (HNSCC) is a prevalent malignancy responsible for approximately 4.6% of global cancer deaths. The standard treatment for locally advanced HNSCC involves cisplatin (CDDP)-based chemotherapy and radiotherapy, which can lead to significant adverse effects, particularly nephrotoxicity.

Objective: Investigated the roles of SNVs *GSTM1, GSTT1, GSTP1* c.313A>G, *XPC* c.2815A>C, *XPD* c.934G>A and c.2251A>C, *XPF* c.2505T>C, *ERCC1* c.354C>T, *MLH1* c.93G>A, *MSH2* c.211+9C>G, *EXO1* c.1765G>A, *TP53* c.215G>C, *CASP3* c.-1191A>G and c.-182-247G>T, *FAS* c.-1378G>A and c.-671A>G, and *FASL* c.-844C>T and kidney function outcomes in HNSCC patients undergoing CDDP treatment.

Methods: A cohort of 109 HNSCC patients was prospectively enrolled. Genotypes were obtained by polymerase chain reaction (PCR). Renal function was assessed using both the chromium-51 labeled ethylenediamine tetraacetic acid (51Cr-EDTA) method and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Results: Patients with isolated *ERCC1* c.354 CT or TT genotypes experienced approximately 8.94% loss of renal function. When these genotypes were combined with the GA or AA genotypes of *MLH1* c.93G>A, renal function loss increased significantly to 18.85% (as measured by CKD-EPI) and 13.47% (by ^{51}Cr-EDTA). Additionally, the decline in renal function was noted to be 17.57% with the combination of *GSTP1* c.313A>G and *ERCC1* c.354C>T and 12.49% with *GSTP1* c.313A>G paired with *MLH1* c.93G>A. The combination of *GSTT1* and *TP53* c.215G>C was linked to a substantial renal function decline of 17.67%, while the pairing of *FAS* c.-1378G>A and *CASP3* c.-1191A>G showed a loss of 11.91%.

Conclusion: Our data indicate, for the first time, preliminary evidence that combined SNVs of CDDP metabolism act as independent prognostic factors and can be used to select patients for personalized treatments that promote renal protection and reduced nephrotoxicity.

Poster Session

P22
Presence of HPV peptides in small extracellular vesicles released by tumor cancer cells in vitro and in vivo

Ł. Ważny[1], M. Gawin[1], M. Gramatyka[1], M. Smolarz[1], D. Kania[1], S. Ludwig[2], N. Ludwig[3], T. Rutkowski[1], P. Widłak[1], T. L. Whiteside[4,5], M. Pietrowska[1]

[1]Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice, Poland
[2]University Hospital Mannheim, Department of Otorhinolaryngology, Head and Neck Cancer Surgery, Mannheim, Germany
[3]University Hospital Regensburg, Department of Oral and Maxillofacial Surgery, Regensburg, Germany
[4]UPMC Hillman Cancer Center, Pittsburgh, PA, United States
[5]University of Pittsburgh School of Medicine, Departments of Pathology, Immunology and Otolaryngology, Pittsburgh, PA, United States

Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is among the most prevalent malignancies worldwide. Despite significant advancements in treatment modalities, the average five-year mortality rate remains approximately 50%, rendering this cancer among the most lethal [1]. The molecular, histopathological, and clinical characteristics differ significantly between HPV(+) and HPV(-) HNSCC. Furthermore, HPV(+) HNSCC exhibits lower mortality rates and reduced resistance to chemotherapy and radiotherapy, resulting in a more favorable prognosis. The molecular mechanisms responsible for these differences remain under investigation. Currently, a potential role of small extracellular vesicles (sEVs), the membrane-bound structures that carrry various biologically active molecules including proteins, lipids, and nucleic acids, in the transport of viral proteins is being investigated. We hypothesize that sEVs derived from HPV(+) cancer cells convey HPV-specific antigens, subsequently inducing a heightened immune response through effective antigen presentation. This mechanism is believed to prime the immune system for cancer eradication.

Methods: Size exclusion chromatography (SEC) was used to isolate sEVs from the supernatant of HPV-positive (SCC-2 and SCC-47) and HPV-negative (PCI-13 and PCI-30) cell lines and from serum samples of patients with HPV(+) and HPV(-) HNSCC. The sEV populations were characteried according to the ISEV guidelines. For LCMS/MS, sEVs were prepared using a modified FASP protocol [2], followed by samples fractionation into the pH 5 and pH 2 peptide fractions. Proteomic analysis was conducted using an Ultimate 3000 RSLC nano-LC system conntected to Q Exactive Plus Orbitrap mass spectrometer. The tolerance for peptide masses was set to 10 ppm and for fragment ion masses to 0.02 Da. Additional confirmation of the presence of viral peptides in sEVs was performed by fluorescence microscopy with co-localization of 3 antibodies conjugated with fluorescent dyes: anti-CD63, anti-HPV16 E7 and anti-HPV16 E2. Ongoing investigations aim to verify the presence of HPV peptides in sEVs isolated from the serum of patients with HPV(+) HNSCC.

Results: We observed substantial differences in the proteomes of HPV(+) and HPV(-) sEVs [3]. Comparison of the raw data with the Swiss-Prot viral database confirmed the presence of viral peptides in the sEVs. Three viral peptides (IKGFEFPHPFPMKPDNTPQFQLTDQSWKSFFER, EIANAAKAIK, VEGDTLADR) were identified in sEVs produced by HPV(+) cell lines, corresponding to three distinct HPV proteins: Replication protein E1, Minor capsid protein L2, and Probable protein E5. This is the first documented instance of HPV peptides in sEVs originating from HPV(+) tumor cells. Current research suggests that E2 and E5 viral proteins, rather than E7/E6, elicit immunogenicity and stimulate antigen-specific T cell responses in HPV(+) individuals.

Conclusions: The simultaneous presence of immunogenic viral proteins and tumor-associated antigens in sEVs suggests that sEV might facilitate both anti-viral and anti-tumor immune responses in patients with HPV(+) HNSCC. The confirmation of the presence of HPV peptides in sEV from plasma of HPV(+) HNSCC patients in ongoing studies will underscore the clinical importance of our findings.

Keywords

Human papillomavirus (HPV); head and neck squamous cell carcinoma (HNSCC); small extracellular vesicles (sEV); immunoregulatory proteins; viral peptides

References

1. Gormley M, Creaney G, Schache A, Ingarfield K, Conway DI. Reviewing the epidemiology of head and neck cancer: definitions, trends and risk factors. *Br Dent J*. 2022;233(9):780-786. doi:10.1038/s41415-022-5166-x
2. Gawin M, Wojakowska A, Pietrowska M, et al. Proteome profiles of different types of thyroid cancers. *Mol Cell Endocrinol*. 2018;472:68-79. doi:10.1016/j.mce.2017.11.020
3. Ludwig S, Marczak L, Sharma P, et al. Proteomes of exosomes from HPV(+) or HPV(-) head and neck cancer cells: differential enrichment in immunoregulatory proteins. *OncoImmunology*. 2019;8(7):e1593808. doi:10.1080/2162402X.2019.1593808

Acknowledgements

We would like to dedicate this work to the memory of Łukasz Skoczylas. The authors express their highest appreciation to Łukasz Skoczylas for his contribution to the research and the resultant findings, acknowledging his exceptional dedication to this area of study.

This research was funded in part by NIDCR grant R01-DE031299 from the National Institutes of Health to LW, MG, TLW and MP.

Poster Session

P23
Trends in epidemiology and prevention strategies for head and neck cancer: Insights from a multinational cohort study

J. Ticks[1]

[1]IHS, Hamburg, Germany

Introduction: Head and neck cancer (HNC) represents a significant global health burden, with rising incidence rates, particularly in developing regions. Understanding the epidemiological trends and effectiveness of prevention strategies is crucial for health policy and resource allocation.

Aim: This study aims to identify current trends in HNC epidemiology, evaluate the effectiveness of preventive measures, and assess diagnostic practices across diverse populations.

Methods: We conducted a retrospective cohort study analyzing HNC cases reported from 2010 to 2022 across five countries (USA, India, Brazil, Germany, and South Africa). Data were collected from national cancer registries, hospital databases, and public health records. We assessed demographic variables (age, sex, ethnicity), risk factors (tobacco use, alcohol consumption, and HPV prevalence), and the impact of vaccination programs on HPV-related cancers. Statistical analyses were performed using multivariate logistic regression to determine the correlation between preventive measures and incidence.

Results: A total of 12,500 HNC cases were identified. The incidence increased by 20% in participants aged 40-60 years, with significant variations across countries ($p < 0.05$). Tobacco use remained the most prevalent risk factor (60%), followed by alcohol consumption (47%) and HPV prevalence (25%) in younger populations. Vaccination efforts in India and Brazil showed a reduction in HPV-related HNC by 15% (95% CI: 10-20, $p < 0.01$) post-vaccination implementation.

Conclusion: This study elucidates critical epidemiological trends in HNC and underscores the importance of targeted prevention strategies. Enhancing public health initiatives, particularly vaccination programs and educational campaigns, could reduce the incidence of HNC, especially among high-risk groups.

Poster Session

P24
Effects of financial strain on quality of life in German head and neck cancer survivors

J. C. Rast[1], V. Zebralla[2], T. Wald[2], A. Dietz[2], G. Wichmann[2], S. Wiegand[1]

[1]Uniklinikum Schleswig-Holstein Kiel, Klinik für Hals-, Nasen-, Ohrenheilkunde, Kopf- und Halschirurgie; Phoniatrie und Pädaudiologie, Kiel, Germany
[2]University Hospital Leipzig, Klinik und Poliklinik für Hals-, Nasen-, Ohrenheilkunde, Leipzig, Germany

Background: A significant financial burden is experienced by survivors of head and neck cancer (HNC) even in Germany, where statutory health insurance is in place. The financial toxicity of cancer can result in elevated morbidity and mortality rates, as well as a reduction in quality of life (QoL). The objective of our investigation is to elucidate the impact of HNC-related financial burden (FB) on QoL, with a view to facilitating a better understanding of the interplay between the two.

Methods: A total of 200 patients, out of the 209 consecutive patients attending the university hospital's cancer aftercare program between August 2022 and March 2023, completed the EORTC QLQ-C30 questionnaire. The data from the quality of life (QoL) scale were analyzed based on the patients' self-reported financial burden (FB). Parametric and non-parametric analyses were employed to evaluate the influence of financial burden (FB) and independent predictors on quality of life (QoL) and QoL scales. Causal diagrams were constructed to illustrate the causal relationship between these variables.

Results: HNC patients reported a notable decline in their quality of life as a consequence of financial burden. Significant detrimental effects of FB were observed on role functioning (RF; p = 0.0011), emotional functioning (EF; p = 0.0039), cognitive functioning (CF; p = 0.0149), and social functioning (SF; p = 0.0011). Advanced stage, advanced T category, and suffering from larynx/hypopharynx cancer demonstrated a significant quantitative interaction with FB, increasing the risk for impaired QoL with respect to RF, EF, CF, and SF.

Conclusion: HNC survivors suffer from significant impaired QoL and FB after treatment. In general, FB impairs particular QoL scales, and these QoL scales are differentially affected by particular tumor characteristics, with FB jointly impairing QoL of HNC survivors.

Poster Session

P26
Detection of methylated tumor markers in head and neck cancer and tumor environment

C. Sust[1], L. Wiehle[1], M. Schmitz[1], N. Häfner[2], F. von Eggeling[2], O. Guntinas-Lichius[2]

[1]oncgnostics GmbH, Research & Development, Jena, Germany
[2]Jena University Hospital, Jena, Germany

Objective: The recurrence rate in head and neck cancers (HNSCC) is high. Alterations in methylation patterns of genes promote the emergence and the recurrence of cancer. One hypothesis is that some cancers relapse due to incomplete resection undetected by standard histopathology. Due to the field cancerization concept, HNSCCs are surrounded by genetic and epigenetic alterations in histologically normal-appearing tissue. The aim of the study is to analyze HNSCC samples upon already established diagnostic DNA methylation tumor markers (HOXA9, ZNF671, ZIC1, PAX6, ZNF833) at the tumor margins and the surrounding tissue. If it is possible to detect these tumor markers close to the negative surgical margins, this could be an indication supporting our hypothesis.

Methods: 15 HNSCC samples were collected during surgery and were frozen immediately. Adjacent sections were used for immunohistochemical staining (Ki-67, AE1/AE3, ASMA). Sections in between were placed on PEN-membrane slides used for laser capture microdissection (LCM). For DNA methylation analyses, methylation-specific real-time PCR (msPCR) upon the previously mentioned tumor markers was performed.

Results: The immunohistochemical staining and msPCR could be well established. As expected, microdissected tumor cells show valid results with high methylation score for the tumor markers, whereas non-tumor cells also show valid, but negative results. Next step is to analyze tissues, with the required morphology for addressing the question of field cancerization effects.

Conclusion: Performing msPCR using cells from LCM for detecting DNA methylation of five tumor markers resulted in high sensitivity and specificity. A clear differentiation between tumor and non-tumor cells could be shown. Results regarding the detection of aberrant methylation in histo-pathologically normal tissue at the resection margins supporting or contradicting the field-cancerization effects will be presented at the congress.

Poster Session

P27
Longterm outcome of patients with early stage glottic or supraglottic cancer – As good as it seems?

T. Wald[1], T. J. Koppe[1], M. Pirlich[1], A. Dietz[1], V. Zebralla[1], V. Kunz[1], M. Stoehr[1], G. Wichmann[1]

[1]Clinic for Otorhinolaryngology, University Hospital Leipzig, Leipzig, Germany

Objectives: Early laryngeal cancer and especially glottic carcinomas show a favorable prognosis after treatment amongst head and neck cancer. However, reports on longterm survival data are rare, often outdated or derived from small cohorts. Tobacco and alcohol consumption are well-known risk factors for the development of early glottic (GC) and supraglottic (SGC) cancer. The influence of other risk factors, e.g. grading, localization or treatment regimen differs in the existing literature and remains unclear.

Methods: Patients with stage I or II glottic or supraglottic (ICD-10-C32.0, C32.1, C32.8 or C32.9) cancer diagnosed and treated at our university hospital from 2007 to 2020 were included in this retrospective study. Survival data and information regarding risk factors were analyzed using univariate and multivariate methods.

Results: 220 patients were included, n = 18 with glottic carcinoma in situ (pTis), n = 165 with early GC and n = 37 with SGC. Mean follow-up (FU) was 64.8 (median FU 59.5; range 1.7-178.2) months. Without pTis, 120-months overall survival (OS) for early GC was 72.7% (mean OS 94.8, 95% confidence interval (CI) 88.6-101.0 months) and for early SGC 56.8% (mean OS 72.3, 95% CI 56.4-88.3 months). In multivariate analyses, risk factors impairing OS were supraglottic localization, tobacco consumption >10 packyears, poor differentiation (grading, G3) of tumor tissue, re-resection after initial R1-resection, definitive radiotherapy and age.

Discussion: Longterm OS in patients with early GC and SGC is mostly favorable with SGC showing inferior OS due to different growth patterns and lymphatic drainage. However, patients at risk need to be identified to prevent them from harm. Precise localization, correct staging, exclusion of mucosal damaging behavior and guideline-conform treatment are important to achieve the best survival for our patients.

Poster Session

P28
Platin-based chemoradiotherapy as definitive treatment in advanced squamous cell carcinoma of head and neck

H. S. V. Sousa[1], V. N. Horita[1], M. Y. Perin[1], D. N. A. Teixeira[1], J. Gruenwaldt[1], E. B. Pereira[1], C. T. Chone[1], G. J. Lourenço[1], L. T. Macedo[1], C. Lima[1]

[1]University of Campinas - UNICAMP, Faculty of Medical Sciences, Campinas, Brazil

Introduction: Patients with unresectable squamous cell carcinoma of head and neck (SCCHN), under organ preservation protocol, or refusing surgery receive platin-based chemoradiation as definitive protocol, and near 40% and 50% of them obtain response to therapy and survive for five years or more, respectively. Nevertheless, the safety and the efficacy of the treatment of SCCHN patients with RT plus cisplatin (CP) or carboplatin (Carbo), administered weekly or every three weeks, is unknown.

Objective: To evaluate toxicities, tumor control, and event-free survival (EFS) and overall survival (OS) of patients with locoregional advanced HNSCC treated with definitive RT with CP or Carbo.

Patients and methods: 233 patients were treated with RT plus weekly or every three weeks CP or Carbo. Response to treatment and toxicity were classified by conventional criteria. Patients´ survival was assessed using Kaplan-Meier method, long-rank test, and univariate and multivariate Cox analyses.

Results: Complete or partial response was seen in 75% of patients, and the distinct protocols did not alter the treatment response. Half of patients presented toxicities grade 3 or 4, being nausea/vomiting and nephrotoxicity more common in RT and CP group and anemia and neutropenia in RT and Carbo group. Two-year EFS and OS probabilities of the total group were 43.3% and 66.0%, respectively. Active smoking, ECOG equal or higher than 2, stage IV tumor, and treatment with RT and Carbo were independent prognostic factors for poorer outcome of SCCHN patients. Patients of these groups had approximately double chance of relapsing and evolving to death than others.

Conclusions: Our data indicate definitive treatment with RT and CP, administered weekly or every three weeks, as the best conventional treatment for unresectable locoregional advanced SCCHN.

Poster Session

P29

Docetaxel and cisplatin induction chemotherapy with or without fluorouracil in locoregionally advanced head and neck squamous cell carcinoma: A real-world data study

M. Y. Perin[1], V. N. Horita[1], D. N. A. Teixeira[1], J. Gruenwaldt[1], E. B. Pereira[1], C. T. Chone[1], G. J. Lourenço[1], L. T. Macedo[1], C. Lima[1]

[1]University of Campinas - UNICAMP, Faculty of Medical Sciences, Campinas, Brazil

Background: Induction therapy (ICT) with docetaxel, cisplatin, and 5-fluorouracil (TPF) or docetaxel and cisplatin (TP) has been indicated for patients with advanced head and neck squamous cell carcinoma (HNSCC), with TPF being more toxic and requiring infusion device or in-hospital administration of 5-fluorouracil.

Objective: To evaluate outcomes of locoregionally advanced patients with HNSCC treated with ICT.

Methods: Toxicity, response rate, and event-free survival (EFS) and overall survival (OS) were evaluated in patients with tumor at stage III or IVA-B (T4 and/or N2b, N2c or N3) treated with TPF or TP.

Results: No significant differences in clinic pathological aspects and treatment response were seen in patients treated with TPF and TP. The median follow-up time was 22.6 months (range: 1.2 to 93.8 months). The two-year and five-year EFS rates of patients of the total group were 33.8% and 25.3%, respectively. At 24 months of follow-up, EFS was lower in patients with ECOG equal or above 1 (18.3% vs. 58.4%, p = 0.001), underweight patients (8.2% vs. 45.3%, p = 0.0001), patients treated with TPF (17.6% vs. 46.4%, p = 0.005), patients who exhibited SD or PD after ICT (0.0% vs. 43.1%, $p < 0.0001$), and patients with interval of 28 days or more between ICT cycles (15.2% vs. 46.3%, p = 0.004) when compared to others (Kaplan-Meier estimates). At 60 months, ESF in TPF was lower than in TP (14.7% vs. 31%, p = 0.005) (Kaplan-Meier estimates). The variables remained predictors of shorter EFS in univariate Cox analysis. Multivariate Cox multivariate analysis showed that patients who exhibited SD or PD, and those experiencing long interval between cycles had 5.56 and 2.79 more chances of presenting tumor progression/recurrence or death from disease than others, respectively.

Conclusion: Our findings indicate TP as a good treatment option for locoregionally advanced HNSCC, especially in socioeconomically limited settings.

Poster Session

P30
Overview of murine head and neck squamous cell carcinoma models for lymphatic and haematogeneous metastasis as well as for primary tumour induction in immunocompetent mice

S. Koerner[1], L. A. Brust[1], F. L. Braun[1], J. P. Kuehn[1], M. D. Menger[2], B. Schick[1], M. Linxweiler[1]

[1]Saarland University Medical Center, Department of Otorhinolaryngology, Homburg, Germany
[2]Saarland University, Institute for Clinical & Experimental Surgery, Homburg, Germany

Objectives: HNSCC is the 6th most common cancer entity worldwide and is associated with a persistently poor prognosis, especially in recurrent and metastatic cases. Hence, valid *in vivo* models are crucial for developing new therapeutic strategies to improve patient outcomes.

Methods: Immunodeficient NOD-SCID mice were inoculated with the human FaDu cell line. Cells were injected into the tip of the tongue for lymphatic metastasis, and in the tail vein for haematogeneous metastasis. Tumor and metastasis development was monitored by calliper measurement and micro-CT, before being finally analyzed by histology after sacrifice of the animals. In a further model, immunocompetent C57BL/6NRj mice were treated with 4NQO in their drinking water for 16 weeks. The induced oral epithelial lesions were resected after 22 weeks, histopathologically characterized and analyzed for intra- and peritumoral immune cell infiltration.

Results: Following a refined experimental protocol, the injection of cells into the tip of the tongue for lymphatic metastasis lead to a manifest tongue tumour with formation of histopathological confirmed cervical lymph node metastases. Furthermore, we could establish a protocol for proper cell harvesting and inoculation into the tail vein of mice. This haematogeneous metastasis model leads to formation of multiple lung metastases. In our immunocompetent mouse model, 4NQO induced multiple epithelial lesions that could be histopathologically identified as invasive squamous cell carcinoma with a modest peritumoral immune reaction.

Conclusion: All murine models described here might be valuable *in vivo* systems for testing future therapeutic approaches in the treatment of HNSCC, with a focus either on anti-proliferative, anti-metastatic and/or immunotherapeutic mode of action. In future studies, we aim to further refine disease monitoring methods using FDG-PET-CT imaging as well as reduce interindividual variability regarding the location and number of metastases.

Poster Session

P31
Investigation of tumor-associated macrophages in different anatomical sites of head and neck cancer

A. Affolter[1], E. Sohn[1], L. Bugia[1], J. Kern[1], N. Rotter[1]

[1]Medical Faculty Mannheim of Heidelberg University, Department of Otorhinolaryngology, Head and Neck Surgery, Mannheim, Germany

Question: Single agent immune checkpoint inhibition has a low objective response rate in head and neck squamous cell carcinoma (HNSCC). Antitumoral M1 and protumoral M2 tumor-associated macrophages (TAMs) regulate immune responses and promote immune evasion and tumor growth. TAMs are associated with a poor outcome in oropharyngeal SCC, but there is little data for other anatomical HNSCC sites. Since there are no robust tumor models on this topic, our study aims to establish one such model.

Methods: The expression of different TAM markers and their precise localization in the tumor/stroma compartments at different sites were assessed by immunohistochemistry and multiplex immunofluorescence. Marker combinations to determine their differentiation were established. For future investigation of TAM activity, we established co-cultures of viable HNSCC *ex vivo* tissue samples with peripheral blood mononuclear cells from the same donor as a novel 3D model.

Results: The expression patterns of macrophage markers such as CD68, CD14, and CD163 were different, indicating heterogeneity between sites but also in tumor samples from the same localization. In 3D cultures, externally added and co-cultured blood monocytes invaded the primary tumor demonstrated by the increased expression of CD45 and CD68 and M2 TAM markers CD206 and CD163, compared to non-co-cultured specimens.

Conclusions: TAMs appear to play a key role in HNSCC and most likely have an impact on prognosis. There was a strong intra- and intratumoral heterogeneity in the expression levels of TAM markers. We will now analyze TAM activity by application of specific novel treatment combinations and correlations to clinical outcomes to gain new insights into the significance of TAMs as a potential target for HNSCC treatment.

Poster Session

P32
Effect of the isolation method on the properties of head and neck squamous cell carcinoma (HNSCC) cells in preclinical models

J. Kern[1], E. Tenschert[1], A. Lammert[1], A. Affolter[1], E. Sohn[1], P. Prohaska[1], N. Rotter[1]

[1]Medical Faculty Mannheim of Heidelberg University, Department of Otorhinolaryngology, Head and Neck Surgery, Mannheim, Germany

Objective: Preclinical models are very important tools for studying tumor biology or response to different therapies such as chemotherapy or immunotherapy. For primary 2D or 3D cell culture models, it is necessary to isolate cells from patient tumors. There are different isolation methods such as enzymatic digestion (ED) or outgrowth culture (Out). The influence of these isolation methods on the cells has not been sufficiently investigated. The aim of this study is therefore to investigate the potential effects of the isolation methods on gene expression and the response to treatment with platinum-based chemotherapeutics.

Methods: Tissue samples from HNSCC patients (n=3) were processed to isolate cancer cells by Out or ED. Cells were expanded in 2D cell cultures up to the second passage and used to build spheroids (25000 cells/spheroid) and to isolate mRNA. The mRNA of the spheroids was isolated after a further 7 days in cell culture and analyzed by nCounter technology (Nanostring Inc.), which allows direct analysis with fluorescently labelled oligonucleotides (100 bases/oligo) that specifically recognize the mRNA of certain genes. A specific cancer panel (Nanostring Inc.) with 750 genes was used. Data analysis was performed by nSolver Analysis Software 4.0. In addition, we analyzed the response of spheroids generated with cells in passages 1, 2, or 3 to treatment with different concentrations of cisplatin (0–50 µM).

Results: Our results show that cells isolated by ED have a different gene expression profile with respect to relevant genes involved in cancer-promoting signaling pathways, such as the EGFR tyrosine kinase inhibitor resistance pathway (e.g., IGF-1R or EGFR), compared to cells isolated by Out. Furthermore, cells isolated by ED are less sensitive to cisplatin treatment than cells isolated by Out.

Conclusion: The isolation method influences the behavior of the cells in preclinical models, which must be taken into account when establishing such models.

Poster Session

P33
Patient derived tumour fragments as pre-clinical ex vivo models for HNSCC

M. Haarmann[1], P. Zimmermann[1], M. Suchan[1], D. Funken[1], R. Thomas[1], J. P. Klußmann[1], J. Brägelmann[1]

[1]University of Cologne, Cologne, Germany

Objective: Studying cancer cells within the patient-specific tumour microenvironment (TME) is crucial for understanding treatment response, as it reveals cellular interactions not observable in in vitro cell culture or cancer organoids. Patient-derived tumour fragments (PDTFs) are a promising pre-clinical model to bridge this gap. Obtained directly from patients, they preserve the spatial and cellular tumour composition, allowing the analysis of tumour behaviour in the context of the TME. In this proof-of-concept study, we explore PDTFs and their potential uses for analysis for head and neck squamous cell carcinoma (HNSCC).

Method: Tumour pieces were derived during routine surgery of HPV-negative HNSCC patients. Close collaboration between clinical and basic researchers ensured an efficient pipeline with minimal time loss. Tumours were mechanically diced into multiple small fragments (~1mm3) and cultured and treated ex vivo for up to 72 hours before analysis.

Result: For this study, 20 patient tumours could be obtained from larynx (n = 7), oral cavity (n = 2), oro- (n = 7) and hypopharynx (n = 4), including 2 relapses and 3 re-biopsies. PDTFs from each tumour were split to treatment with cisplatin (n = 15 tumours), nivolumab (n = 4 tumours) or vehicle. In total, RNA from PDTFs of 13 tumours could be extracted to be subjected to qPCR, with 6 of these passing QC for low input RNA sequencing.

Results from qPCR and RNA-seq showed overall comparable treatment effects between the different tumours and assays. Transcriptomic changes were in line with patterns observed in established HNSCC cell lines and indicative for effects on both, tumour cells and leukocytes.

Conclusion: Our study demonstrates that PDTFs may serve as a pre-clinical ex vivo model for investigating specific tumour biology within the full TME, providing valuable insights into treatment effects on both, the tumour and surrounding leukocytes in HNSCC.

Poster Session

P34
Development of an orthotopic *in vivo* model for the combination of therapeutic vaccination with low-dose irradiation in HPV-driven oropharyngeal cancers

J. Audouze-Chaud[1], K. van der Gouw[1], A. K. Schlosser[1], C. Schifflers[1,2,3], S. Zottnick[1,2], A. Riemer[1,2]

[1]German Cancer Research Center (DKFZ), Immunotherapy and Immunoprevention, Heidelberg, Germany
[2]German Center for Infection Research (DZIF), Heidelberg, Germany
[3]University of Namur, Cellular Biology Research Unit (URBC), Namur Research Institute for Life Sciences (NARILIS), Namur, Belgium

HPV-driven oropharyngeal cancer has displayed rising incidence over the past years. Current treatment options such as radiotherapy or chemotherapy are not always efficient. Therapeutic vaccination represents an attractive alternative for an effective treatment with low side effects. More and more evidence suggests that the tumor microenvironment, and in particular immune microenvironment, plays a key role in the poor response to conventional treatments, stressing the need for a strong *in vivo* model. Most of those current *in vivo* models fail to recapitulate human conditions, as they are either not strictly dependent on HPV and/or are not using immunocompetent humanized mice, thereby lowering the chances of developing a clinically successful treatment. In order to test our therapeutic vaccine in a realistic setting, we established a novel HPV16 tumor model in MHC-humanized A2.DR1 mice For this purpose, primary lung cells from A2.DR1 mice were isolated and immortalized by transduction with HPV16 oncoproteins E6 and E7. In order to make them tumorigenic and trackable, cells were then transfected with a plasmid encoding HRAS and firefly luciferase. The newly generated tumor cells were then injected to the base of the tongue. This orthotopic *in vivo* model mirrors the tumor immune microenvironment better than a subcutaneous injection. In addition, A2.DR1 mice possess human HLA alleles, allowing for a better modelling of human antigen presentation, Finally, the tumor cells have been transduced with HPV16 oncoproteins, making them truly HPV dependent. This model is therefore well suited for the study of therapeutic approaches for HPV-driven oropharyngeal cancer *in vivo*. Additionally, a negative counterpart to the E6/E7-dependent orthotopic tumor model is currently being generated. Taken together, these experiments will be a crucial step in the preclinical assessment of new therapeutic HPV vaccine combination treatment strategies to effectively combat mucosal tumors.

Poster Session

P35
Combining head and neck cancer tissue slice cultures with sequential immunofluorescence for functional drug testing

A. I. Pusztai[1], K. Obermeier[2], I. Dewenter[2], P. Jurmeister[1], K. Sharaf[3], S. Otto[2], F. Klauschen[1], A. Mock[1]

[1]Institute of Pathology, LMU, Munich, Germany
[2]Ludwig Maximilian University of Munich, Department of Oral and Maxillofacial Surgery and Facial Plastic Surgery, Munich, Germany
[3]Ludwig Maximilian University of Munich, Department of Otorhinolaryngology, Munich, Germany

Objective: Organotypic tissue slice cultures (TSCs) could be shown to faithfully recapitulate the tumor microenvironment for up to a week. However, conventional cytotoxic assays are of limited use to assess the mode of actions of drugs in TSCs. To this end, we investigated the potential of sequential immunofluorescence (seqIF) to study the functional changes in both the tumor and immune cell compartment upon drug treatment.

Methods: Fresh tumor tissue of head and neck squamous cell carcinomas (HNSCC) was obtained directly from the operating room following tissue processing within 1-2 hours. The tissue was embedded in agarose and cut into 300 µm thick slices using a vibrating microtome (Compresstome VF-310-OZ, Precisionary) and cultivated at the air-liquid interface. Drug testing was performed between 24h and 72h after cultivation start and the tissue consecutively formalin-fixed and paraffin-embedded. SeqIF was performed with the COMET system (Lunaphore). Data analysis was conducted by Horizon viewer (Lunaphore) and downstream R functions.

Results: Viable HNSCC tissue slice cultures could be cultivated for up to a week. There was no significant viability benefit in using fetal bovine serum as measured by Caspase 3 staining. Tissue microarrays were found to be of disadvantage in tissue processing for seqIF. So far, a total of 25 antibodies could be successfully established and costained by seqIF for biological readout. The first drug tested in this model system was the JAK inhibitor ruxolitinib demonstrating e.g. a significant downregulation of pSTAT3 upon treatment. A more thorough readout is currently under investigation.

Conclusions: We could successfully combine organotypic tissue slice cultures of HNSCC with seqIF enabling functional drug testing with phenotypic readouts on the single cell resolution. Here we aim to include more drugs and benchmark this method by comparative spatial transcriptomics analysis.

Poster Session

P36
Establishment of differentiation therapies targeting squamous cell carcinomas of the head and neck and other locations

F. Oppel[1], S. Gendreizig[1], L. Hose[1], S. Shao[1], F. Brasch[2], L. U. Scholtz[1], I. Todt[1]

[1]Bielefeld University, HNO Klinik, Bielefeld, Germany
[2]Klinikum Bielefeld, Department of Pathology, Bielefeld, Germany

Head and neck squamous cell carcinoma (HNSCC) is a highly malignant disease. The 5-year overall survival rate remains substantially unaltered for decades, so new therapy approaches are urgently needed. Our group has recently characterized the terminal differentiation of HNSCC cells to tumor corneocytes. Our differentiation model using primary HNSCC cell cultures demonstrated a striking loss of malignancy upon transplantation into immunodeficient mice. RNA-seq and ATAC-seq confirmed differentiation by cornification, wound healing and inflammatory signaling. This was accompanied by chromatin remodeling and an overall closure of the genome, a known sign of cell differentiation in stem cell systems. Using FFPE tissue, we verified these results by immunofluorescence and discovered a common differentiation program in HNSCC and normal mucosa. In addition, we detected that cytokeratin 17, representing a basal stem cell marker in normal mucosa, was upregulated during HNSCC cell differentiation and is a suitable biomarker of this process. Interestingly, cornification was detected to occur reproducibly surrounding necrotic tumor tissue, indicating an impact of inflammatory stimuli. Accordingly, we showed that this process can be triggered in HNSCC cells by the treatment with inflammatory cytokines, leading to increased cell adhesion and upregulation of differentiation markers. Moreover, we hypothesized that squamous cell carcinomas (SCCs) in general may be more suitable for differentiation therapies than other tumor types, as their normal differentiation path leads to cell death by cornification. A broad investigation of FFPE tissue samples of SCCs of the skin, penis, vulva and cervix revealed differentiation dynamics similar to HNSCC tissue. This indicates that a targeted differentiation therapy could interfere with HNSCC cell malignancy and might furthermore be applicable throughout distinct SCC types in the human body. This should be elaborated in future investigations.

Poster Session

P37
Propagator methods for survival analysis

J. Schlecker[1,2], I. Kurth[1,3,4], W. W. Hadiwikarta[1,3,4]

[1]German Cancer Research Center (DKFZ), E220 Radiobiology/Radiooncology, Heidelberg, Germany
[2]University of Heidelberg, Department of Physics and Astronomy, Heidelberg University,Department of Physics and Astronomy, Heidelberg, Germany
[3]National Center for Radiation Research in Oncology (NCRO), Heidelberg Institute for Radiation Oncology (HIRO), Heidelberg, Germany
[4]German Cancer Consortium (DKTK), Core Center Heidelberg, Heidelberg, Germany

To address a number of shortcomings of conventional survival analysis methods such as Kaplan-Meier Curves and Cox Regression techniques, we introduce a novel survival model inspired by Markov Chain and Stochastic Process models. By conceptualising the patient as a particle moving stochastically within a compound state-space of discrete and continuous variables that represent the disease state at any given time, we apply methods from Quantum and Statistical Physics to describe the patient"s trajectory in this space.

This model introduces randomness as a conceptual result of the dynamics of obscured variables, offering distinct interpretability. Regression parameters have direct meaning in terms of generalised forces acting on the patient. Moreover, assumptions are made at a patient-near level, enhancing clarity in what is required for applicability. Furthermore, this new model provides a more detailed interpretation of the shape of survival curves, naturally considering interactions between parameters. In the end, we aim to aid treatment strategy decisions in application of this survival model. We present the conceptual framework and construction of this model, emphasizing how it deals with perceived "randomness".

P38
Evaluating the CAM assay as a preclinical model for head and neck tumors: Engraftment efficiency, xenografts and serial transplantation

T. Kleitke[1], L. Eichhorst[1], C. Maletzki[2], A. S. Becker[3], R. Mlynski[1], D. Strüder[1]

[1]University, Department for Otorhinolaryngology, Head and Neck Surgery 'Otto Körner', Rostock, Germany
[2]University, Medical Clinic III for Hematology, Oncology, and Palliative Medicine, Rostock, Germany
[3]University, Institute for Pathology, Rostock, Germany

Introduction: Due to heterogeneity in biology, etiology, and phenotype, head and neck squamous cell cancer (HNSCC) is challenging to treat and associated with a poor prognosis. The chorioallantoic membrane (CAM) assay has become popular, to gather data before moving to more complex mammalian studies. However, its utility is limited by insufficient standardization. This study aims to achieve reproducible CAM engraftment rates of established, patient-derived cell lines and patient derived xenografts.

Materials & Methods: We examined the engraftment rate of established cell lines UTSCC14 and UTSCC15, transfected cell line PECA15J/NIR and patient-derived cell lines HNSCC16 and HNSCC48 in the CAM assay. PDX samples from our biobank were implanted as 3x3x3 mm, 1x1x1 mm fragments or cell strained samples. The CAM was either lacerated, conditioned with ethanol or left untreated. After a 7-day incubation period, the viability of the samples was evaluated.

Results: Engraftment rates for cell lines were 74% to 83%. The biobank PDX engraftment rate was 37,5% (6/16) for 3x3x3 mm fragments and 40% (6/15) for 1x1x1 mm fragments. Serial transplantation of PECA15J/NIR tumors yielded an engraftment rate of 60% (12/20). In a comparative study using a mouse model, engraftment rates of 36% were determined for surgical resection specimens. CAM laceration (62%, 13/21), ethanol treatment (29%, 5/17) and tumor homogenization (33,3%, 8/24) did not improve engraftment rates.

Discussion: The CAM assay is an efficient model for PDX generation with engraftment rates comparable to mouse models. The CAM preparations did not enhance engraftment rates, indicating the need for further optimization to establish the CAM assay as a standard model for PDX generation.

Poster Session

P39
Identification of distinct subpopulations of cancer associated fibroblasts in oral squamous cell carcinoma by imaging mass cytometry

S. Tornaas[1], D. Kleftogiannis[1,2], S. Fromreide[1], H. Ytre-Hauge Smeland[3,1], H. J. Aarstad[4], O. K. Vintermyr[3], L. A. Akslen[3,1], H. Nitin Dongre[1], D. E. Costea[3]

[1]University of Bergen, Center for Cancer Biomarkers CCBIO and Gade Laboratory for Pathology, Bergen, Norway
[2]University of Bergen, Computational Biology Unit, Department of Informatics, Bergen, Norway
[3]University of Bergen/Haukeland University Hospital, Pathology, Bergen, Norway
[4]Haukeland University Hospital, Department for Ear-Nose-and-Throat, Head and Neck Clinic, Bergen, Norway

Background: Although cancer associated fibroblast (CAF) heterogeneity is recognized in oral squamous cell carcinoma (OSCC), the phenotype and role of CAF subpopulations in OSCC is still unknown.

Objectives: This study aimed to dissect CAF heterogeneity and spatial location by using imaging mass cytometry on formalin fixed paraffin embedded (FFPE) tissues from primary OSCC.

Methods: Imaging mass cytometry (Hyperion technology) was used to identify 24 markers (E-cadherin, EGFR, Ki-67, α-SMA, CD140β, FAP, FSP-1/S100A4, CD90/Thy-1, integrin α-11, collagen-1, tenascin-C, caveolin-1, CD4, CD8a, CD16, CD20, CD68, CD163, FoxP3, granzyme B, CD31, podoplanin, CD146, and YAP-1) in HPV-negative OSCC lesions (n=10). Tissue images were segmented employing the Steinbock framework. Unsupervised clustering of single-cell data was carried out using a bioinformatics pipeline developed in R language.

Results: Unsupervised clustering identified several major cell types, with E-cadherin+ cells being the most abundant (30%) and CD146+ being the least abundant cell type (0.62%). Among the immune component, CD4+ cells were most abundant (11.72%) followed by CD8+ cells (10.6%) and CD68+ cells (10.25%). The cells negative for other major lineage markers and positive for stromal markers were considered as CAFs and they clustered in 6 clusters defined as CAF1 to CAF6. CAF1-3 were high in FAP and CD140β while CAF 4-6 were low expressing FAP and CD140β. CAF1 and 6 expressed high αSMA, while CAF 3 and 5 expressed high collagen-1. CAF6 had high expression of integrin α-11. CD140β and CD90) and CAF-2 (high FAP and collagen-1). CAF1 and 3 subtypes were found closer to immune cells whereas CAF2 and 6 were closely located to cancer cells.

Conclusions: We identified six distinct populations of CAFs in OSCC by using imaging mass cytometry. Their clinical implications can be further revealed by using this method on larger patient cohorts with FFPE tissues available in diagnostic biobanks.

Poster Session

P40
Characterization of irradiated mucosa using confocal laser endomicroscopy in the upper aerodigestive tract

M. Goncalves[1], L. Thesing[2], M. Sievert[3], B. Akhanda Panuganti[4], A. Scherzad[1], T. Meyer[1], S. Hackenberg[1]

[1]Universitätsklinikum Würzburg, Klinik und Poliklinik für Hals-Nasen-Ohrenheilkunde, Kopf- und Hals-Chirurgie, Würzburg, Germany
[2]RWTH-Aachen University, Aachen, Germany
[3]Friedrich-Alexander-Universität Erlangen-Nürnberg, Department of Otorhinolaryngology, Head and Neck Surgery, Erlangen, Germany
[4]Washington University in St. Louis, Department of Otolaryngology-Head and Neck Surgery, St. Louis, MO, United States

Objective: Confocal laser endomicroscopy (CLE) enables real-time in vivo optical mucosa biopsy in the upper aerodigestive tract. Previous studies demonstrated its potential in identifying malignant tissue, but none examined mucosa treated with radiotherapy. This study characterizes the appearance of irradiated mucosa using CLE.

Methods: We recorded 58 CLE sequences (860 seconds, 6,884 frames) in 10 patients previously treated with radiotherapy for upper aerodigestive tract tumors. A corresponding tissue biopsy (formalin-fixed, H&E stained) was taken as the reference standard for each sequence. We analyzed each sequence regarding differences from physiological mucosa and characterized irradiated mucosa in CLE.

Results: Irradiated mucosa in CLE exhibits irregular tissue architecture. Radiation induces DNA damage, apoptosis, and tissue inflammation, leading to hyperkeratotic and fibrotic tissue. Consequently, CLE showed a wider range of cell morphology and tissue structure than physiological mucosa. In addition to regular honeycomb-like patterns, the tissue displayed uneven, blurry, and cell-rich areas. Irradiated mucosa appears more irregular and variable in CLE than radiation-naïve mucosa.

Conclusion: Irradiated mucosa can be differentiated from healthy mucosa in CLE. When applying CLE to irradiated mucosa, it should be considered that it has a higher baseline diversity of appearance than physiological mucosa. Further studies are needed to evaluate its impact on tumor detection with CLE in irradiated mucosa.

Poster Session

P41
Double Trouble: Identifying optimal combinations for the IAP-inhibitor Debio 1143 for the radiosensitization of HNSCC cell lines and tissue slices

J. Röhrle[1], F. Gatzemeier[1], C. Petersen[1], K. Rothkamm[1], C. Betz[1], T. Rieckmann[1], M. Kriegs[1]

[1]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Question: Radiochemotherapy with the apoptosis stimulator Xevinapant was the first arm to ever demonstrate superiority over radiochemotherapy in a randomized (phase 2) trial in HNSCC. However, the following phase 3 Trilynx-study recently failed to achieve its primary endpoint. In this project we aim at identifying effective combinations for Xevinapant to optimize curative treatment for HPV-negative HNSCC patients by testing an ATR (Tuvusertib) and a PARP inhibitor (Olaparib) in comparison to cisplatin.

Methods: Effectiveness of ATR-inhibition was tested through the potential to inhibit radiation-induced G2 cell-cycle arrest. Cytostatic effects were assessed by proliferation assay, radiosensitization by colony formation assay. Apoptosis induction was assessed by Annexin V staining. We used 4 radioresistant HPV-negative cell lines and clinically achievable doses of the inhibitors.

Results: Effective inhibition of radiation-induced G2-arrest was already observed at 30nm Tuvusertib in all cell lines, Xevinapant and Olaparib were used at a moderate dose of 1µM. Proliferation analyses showed varying sensitivities towards the different inhibitors. With the exception of HSC4, growth inhibition under combined treatment did not suggest more than additive effects. When combined with radiation in colony formation assays, our current data suggest the most effective radiosensitization through ATR-inhibition and the least through Xevinapant. So far, combining Xevinapant with the other agents mostly suggested additive instead of synergistic effects. Xevinapant and irradiation resulted in increased rates of apoptosis, moderately increased under combined inhibition.

Conclusions: ATR-inhibition resulted in the most distinct radiosensitization. Our current data suggest a mostly moderate benefit for combinations with Xevinapant. The contribution of the programmed cell death pathways apoptosis and necroptosis will be further be investigated.

Poster Session

P42
Diagnosis of lymph node metastases in head and neck cancers using Hsp70-specific fluorescence imaging

J. L. Wolf[1,2,3], K. L. K. Holzmann[1,2,3], B. Wollenberg[1], G. Multhoff[2,3], M. Wirth[1]

[1]Department of Otorhinolaryngology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany
[2]Institute for Radiotherapy and RadioOncology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany
[3]Central Institute for Translational Cancer Research, Technical University of Munich (TranslaTUM), Munich, Germany

Objective: Cervical lymphadenectomy is often associated with side effects and a reduced quality of life. In this study, we assess the potential of fluorescence imaging using the tumor-specific tracer "TPP-IRDye800" targeting membrane-bound Hsp70 on cancer cells, to image tumor-involved lymph nodes (LNs). Heat shock protein 70 (Hsp70) is frequently upregulated in the cytoplasm and presented on the membranes of many tumor entities, including head and neck squamous cell carcinomas (HNSCC). Previous studies could show that TPP effectively distinguishes tumor tissue from healthy tissue and delineates tumor margins.

Materials and Methods: TPP-IRDye800 was topically applied on both positive and negative lymph nodes of 15 patients with different entities of head and neck cancers. The emitted signal was imaged with an EM-CCD camera and also evaluated using a clinical imaging system (Figure 1). Tumor specificity was confirmed by immunohistochemistry and the signal-to-background ratio (SBR) was measured. Tumor cell-spiked swine lymph nodes were used to assess the sensitivity of the TPP tracer.

Results: TPP-IRDye800 demonstrated a clear delineation of histologically confirmed lymph node metastases in fresh tissues and cryosections through an enhanced signal-to-background ratio. The SBR is mainly in the range of 2-3. Notably, fluorescence imaging revealed patterns that closely corresponded to the histological findings, further validating the tracer's specificity (Figure 2). A positive fluorescence signal could be detected with less than 50,000 mHsp70-positive tumor cells spiked in the swine lymph nodes.

Conclusion: TPP-based fluorescence imaging shows promise for pathological identification of lymph node metastases and holds potential for future surgical applications. Future studies will explore the intravenous applications of the TPP-IRDye800 tracer in HNSCC patients.

Figure 1: Comparing pos. and neg. LNs using a clinical imaging system

Figure 2: Characterizing LN metastases

Fig. 1

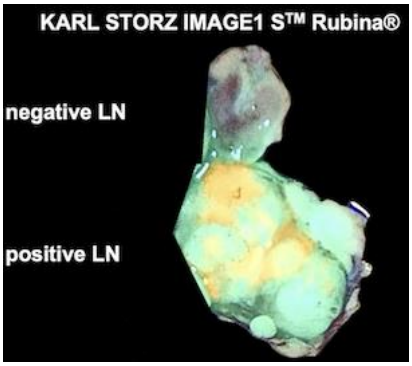
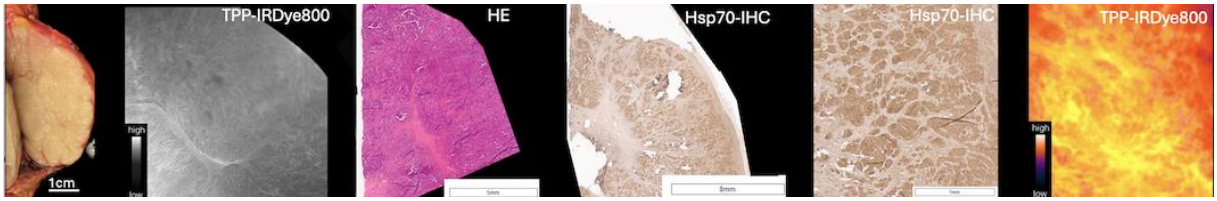

KARL STORZ IMAGE1 S™ Rubina®
negative LN
positive LN

Fig. 2


TPP-IRDye800
1cm
high
low
HE
Hsp70-IHC
Hsp70-IHC
TPP-IRDye800
high
low

Poster Session

P43

Implications of the partial volume effect correction on the spatial quantification of hypoxia and clonogenic cell density based on [18F]FMISO and [18F]FDG PET/CT data

A. Kafkaletos[1], I. Sachpazidis[1], M. Mix[2], M. Carles[3], H. Schäfer[4], A. Rühle[5], N. H. Nicolay[5], M. Bock[6], M. Lazzeroni[7], I. Toma-Dasu[7], A. L. Grosu[4], D. Baltas[1]

[1]Universitätsklinikum Freiburg, Department of Radiation Oncology, Division of Medical Physics, Freiburg, Germany
[2]Universitätsklinikum Freiburg, Department of Nuclear Medicine, Freiburg, Germany
[3]La Fe Health Research Institute, Valencia, Spain
[4]Universitätsklinikum Freiburg, Department of Radiation Oncology, Freiburg, Germany
[5]University Hospital Leipzig, Department of Radiation Oncology, Leipzig, Germany
[6]Universitätsklinikum Freiburg, Department of Radiology, Division of Medical Physics, Freiburg, Germany
[7]Karolinska Institute, Oncology-Pathology Department, Stockholm, Sweden

Objective: The quantification of hypoxia and estimation of the clonogenic cell density (ρ) are crucial for personalized radiotherapy. This study evaluates the impact of partial volume effect (PVE) correction on [18F]fluoromisonidazole (FMISO) PET images for quantification of hypoxia via partial oxygen pressure (pO2) maps and on [18F]fluorodeoxyglucose (FDG) for the estimation of cell density in the gross tumor volume (GTV).

Methods: FMISO and FDG PET images from head and neck cancer cases were corrected for PVE using a recovery coefficient based method. The standardized uptake value (SUV) maps of the FMISO images were converted to pO2. The pO2 distributions from corrected and uncorrected data were evaluated against published polarographic data. A pO2 threshold of 10 mmHg was used to delineate the hypoxic tumor volume (HTVpO2) which was compared to the HTV, defined by the conventional tumor-to-muscle ratio (TMR) method (HTVTMR). The FDG SUV maps were normalized using a reference region of healthy muscle tissue. The corrected and uncorrected FDG SUV maps were converted to ρ by a linear equation. The corrected and uncorrected FDG SUV distribution and subsequent ρ maps were compared.

Results: The application of the PVE correction decreased the minimum pO2, increased HTVpO2 and led to the detection of more hypoxic cases (HTV>0). The pO2 distribution improved aligned better with published polarographic data. The HTVTMR and HTVpO2 became comparable only after the application of correction, although high interpatient variability was observed. The correction of the FDG images caused an average increase of 70% in SUVmax and 44% in SUVmean across the population, while the resulting ρmean was decreased on average by 13%.

Conclusions: PVE correction is recommended before converting SUV to pO2 for the spatially resolved quantification of hypoxia, while further investigation is required to evaluate the effect of PVE correction on the discretized determination of cell density.

Poster Session

P44
Molecular imaging-guided radiotherapy of the head and neck has the potential to enhance treatment tolerability

F. Stritzke[1], K. Dvornikovich[1], H. Lau[1], P. Schröter[1], S. Regnery[1], L. Bauer[1], K. Weusthof[1], J. Debus[1], T. Held[1]

[1]Heidelberg University Hospital, Department of Radiation Oncology, Heidelberg, Germany

Objective: In head and neck cancer, accurate target delineation is crucial for the efficacy and tolerability of radiotherapy. Advancements in diagnostic sensitivity, such as fluorodeoxyglucose positron emission tomography (FDG-PET), have altered the cost-benefit calculation of treating the apparently uninvolved neck. Non-randomized evidence suggests that reducing the volume and radiation dose to the elective neck is oncologically safe. However, a direct comparison of the potential benefits has not been performed. Therefore, we compared the dosimetric parameters and complication probabilities of conventional vs. FDG-guided radiotherapy.

Methods: We identified 17 head and neck cancer patients with FDG-PET/CT staging, treated with definitive radio(chemo)therapy. For FDG-guided radiation (Fig. 1A), we restricted the elective planning target volume (PTV) to 2 cm craniocaudal from the gross tumor and pathological FDG uptake. The dose to low-risk areas was reduced from 56 Gy to 46 Gy. Instead of the simultaneous integrated boost used in conventional plans, the boost was deployed sequentially in FDG-guided radiation plans.

Results: FDG-guided radiation substantially reduced the elective PTV (Fig. 1B). This adaptation led to an average decrease of 5.5 Gy in the mean dose to the swallowing apparatus (p=0.01; Fig. 2A). According to an established complication probability model, FDG-guided radiation would significantly mitigate the median risk of experiencing persistent dysphagia from 24% to 19% (p=0.04; Fig. 2B).

Conclusion: Adapting radiotherapy to the advanced sensitivity of the FDG-PET significantly reduces radiation dose to critical structures, decreasing the risk of persistent dysphagia. This approach will be evaluated in the randomized clinical trial MIGNITE.

Fig. 1

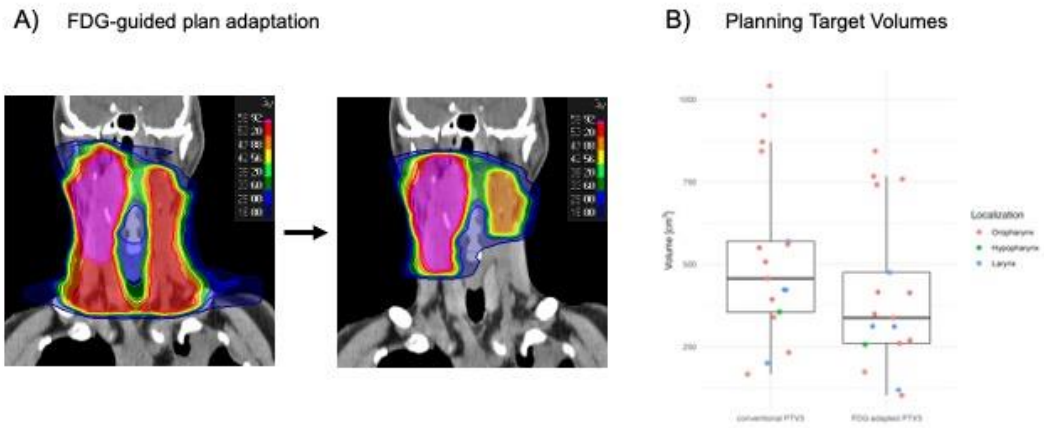


Fig. 1. Adaptation of treatment plans based on FDG-PET imaging. (A) Comparison of conventional and FDG-guided radiotherapy plans for a patient with oropharyngeal cancer. (B) Reduction of the planning target volume through adaptation by disease site.

Fig. 2

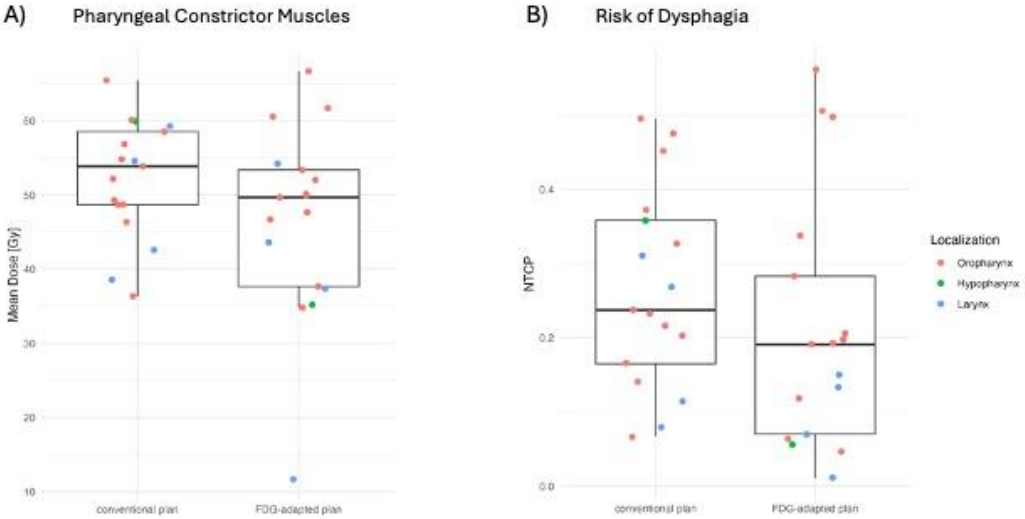


Fig. 2. FGD-guided radiotherapy may improve swallowing function. (A) The mean dose to pharyngeal constrictor muscles in conventional and FDG-guided radiotherapy plans. (B) Individual risk of swallowing dysfunction 6 months after radio(chemo)therapy, calculated by the model published by Cristianen et al., 2011. Statistical analyses were performed using the Wilcoxon signed-rank test.

Poster Session

P45
Evaluation of auto-segmentation solutions for lymph node level delineation in head and neck cancer radiation therapy

K. Dvornikovich[1], D. Neugebauer[1], F. Wagner[1], F. Stritzke[1], S. Regnery[1], L. Bauer[1], P. Schröter[1], K. Weusthof[1], H. Franke[1], J. Debus[1], T. Held[1]

[1]Heidelberg University Hospital, Radiation Oncology, Heidelberg, Germany

Objective: The aim of this study was to evaluate the use of commercial auto-segmentation solutions for treatment planning in radiation therapy for patients with head and neck cancer.

Methods: For 62 head and neck cancer patients, the physician-delineated low risk clinical target volume (CTV3) was divided into individual lymph node levels (ground-truth) [1] and corresponding auto-segmented lymph node levels were created using MVision and Limbus. The structure sets were compared regarding geometric agreement of individual lymph node levels and composite target volumes.

Results: Most common tumor sites were the oropharynx (56.5%), larynx (19.4%) and nasopharynx (12.9%). Nodal metastases were detected in 42 (67.7%) patients.

For the individual lymph node levels, median Dice Similarity Coefficients (DSC) ranged from 0.38-0.77 (MVision) and from 0.13-0.73 (Limbus). Median Hausdorff Distances (HD, [cm]) were between 1.1 and 2.2 (MVision) and 1.3 and 2.7 (Limbus).

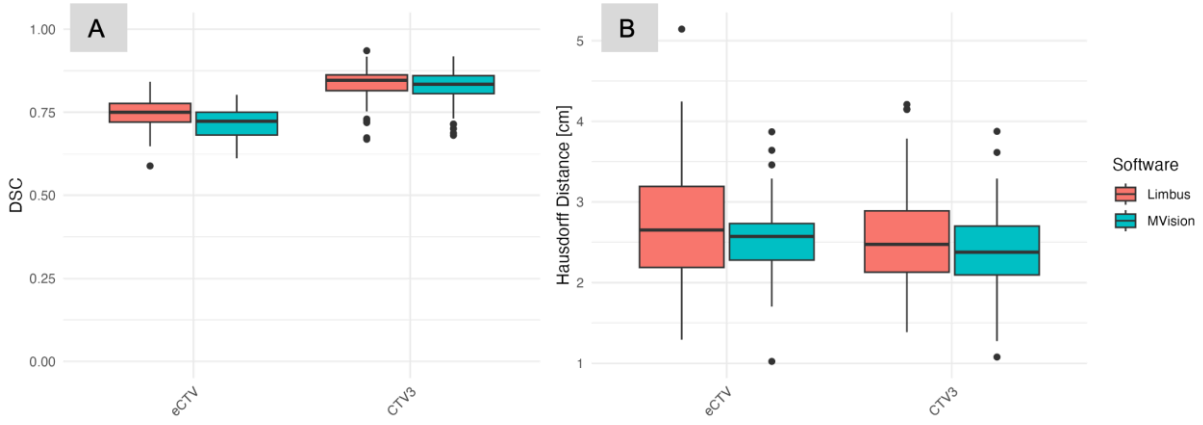
DSCs had a median of 0.72 (MVision, range 0.61-0.80) and 0.75 (Limbus, range 0.59-0.84) for elective nodal clinical target volumes (eCTV), and 0.83 (MVision, range 0.68-0.92) and 0.85 (Limbus, range 0.67-0.94) for CTV3s (Fig. 1a). Median HDs [cm] were 2.6 (MVision, range 1.0-3.9) and 2.7 (Limbus, range 1.3-5.1) for eCTVs, and 2.4 (MVision, range 1.1-3.9) and 2.5 (Limbus, range 1.4-4.2) for CTV3s (Fig. 1b).

Conclusion: Auto-segmentation of lymph node levels offers promising potential for increasing efficiency and consistency. Using the auto-segmented contours, machine-learning-based plans will be created, and the clinical usability will be further evaluated as part of an expert rating.

[1] Grégoire (2014) Radiother Oncol

Fig. 1: DSC: Dice Similarity Coefficient; eCTV: elective nodal clinical target volume; CTV3: low risk clinical target volume

Fig. 1


A
B
DSC
1.00
0.75
0.50
0.25
0.00
Hausdorff Distance [cm]
5
4
3
2
1
eCTV
CTV3
Software
Limbus
MVision