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MEETING ABSTRACTS Open Access

Abstracts of the Annual Conference of the German Society for Biological Radiation Research (DeGBS)

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Welcome Address

Dear colleagues, Dear friends of the DeGBS,

It is our great pleasure to welcome you to the 2025 Annual Conference of the **German Society for Biological Radiation Research (DeGBS)**, held this year in the beautiful city of Munich. As in previous years, our meeting brings together a diverse and interdisciplinary community of scientists committed to advancing our understanding of biological responses to ionizing radiation and improving radiotherapeutic strategies.

The scientific program reflects the dynamic and multifaceted nature of our field. It is structured into four thematic sessions and one dedicated **Young Investigators' Session**:

Session A explores the fundamental mechanisms of radiation responses, including DNA repair and cell fate decisions.

Session B focuses on signaling and adaptive responses, covering signal transduction, epigenetics, radiation qualities, temporal and spatial fractionation effects.

Session C addresses cell-extrinsic and systemic modulators, such as cell-to-cell interactions, microenvironmental and immune-related aspects.

Session D, a joint session with the **German Society for Radiation Oncology (DEGRO)**, highlights translational innovations in radiotherapy, including personalized and combined modality treatment approaches, predictive and prognostic marker constellations.

Session E, our **Young Investigators' Session** entitled *"Replace, Reduce, Refine"*, showcases innovative and responsible strategies in translational radiation research, emphasizing the next generation of scientific leadership.

We are excited about the outstanding contributions submitted to this meeting and look forward to stimulating scientific discussions, new collaborations, and a shared vision for the future of biological radiation research.

On behalf of the **DeGBS**, we thank you for your participation and wish you an inspiring and enjoyable meeting.

With kind regards,

The Organizing Committee

Kirsten Lauber (LMU University Hospital München)
Anna Friedl (LMU University Hospital München)
Horst Zitzelsberger (Helmholtz Center München)

Session A: Fundamental mechanisms of cellular responses to radiation

a) Key note lecture

A-K1

Mitochondria at the hub of cell death and immunity in irradiated cancer cells

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Radiation Oncology 2025, **20(s1)**:A-K1

Mitochondrial integrity controls cellular and immunological homeostasis in various pathophysiological settings. Previous work from our lab demonstrated that the cytosolic accumulation of mitochondrial DNA (mtDNA) in irradiated breast cancer cells is paramount for their ability to secret type I interferon (IFN) upon CGAS-STING signaling, hence initiating targeting-tumor immunity. Such a release is mediated by the molecular machinery for mitochondrial outer membrane permeabilization (MOMP), hence being actively inhibited by the anti-apoptotic protein BCL2. Moreover, the proficient disposal of permeabilized mitochondria by autophagy (a process that is commonly known as mitophagy), as well as the MOMP-driven activation of apoptotic caspases, tonically inhibits type I IFN responses elicited by radiation therapy. As general autophagy is also fundamental for the optimal release of immunostimulatory ATP by cancer cells undergoing immunogenic cell death (ICD) we are focusing on strategies that would maximize type I IFN secretion in the context of preserved ATP release to obtain superior immunostimulatory effects by radiation therapy in HR + breast cancer.

b) Proffered papers—oral presentation

A-O1

Multi-modal computational analyses reveal motifs of interferon signaling and DNA repair as hallmarks of the radiation response in HNSCC

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Radiation Oncology 2025, **20(s1):**A-O1

Background Radiotherapy is a standard treatment for head and neck squamous cell carcinoma (HNSCC), yet resistance remains a major challenge. Accordingly, investigating the molecular mechanisms underlying radiation resistance and their prognostic implications in clinical cohorts is a major focus of both basic and clinical research.
Materials and methods We subjected a panel of established HNSCC cell lines to single-cell RNA sequencing and employed Non-Negative Matrix Factorization (NMF) to extract gene expression programs reflecting heterogeneity. Among the cell lines, Cal33 exhibited the greatest intracellular heterogeneity, accompanied by pronounced radioresistance. SKY-FISH confirmed the presence of genetically distinct subclones within this line. We established 10 Cal33 sublines with varying degrees of radiation resistance, serving as an informative model system to study intra-tumor heterogeneity and post-radiation cell fate decisions.
Results Transcriptional similarity between Cal33 sublines did not correlate with radiation resistance, suggesting that mechanisms beyond the major transcriptional determinants drive resistance. DNA damage repair motifs were associated with increased resistance, while high basal levels of interferon (IFN) signaling were linked to increased radiosensitivity. Time-resolved mass cytometry confirmed IFN signaling in the sensitive subline and DNA repair activation in the resistant subclone post-radiation. Chk1 inhibition elevated STAT1/STAT3 activation upon irradiation, revealing the causal interconnection between DNA repair and IFN signaling. Functionally, ATM inhibition—rather than IFNβ treatment—sensitized the sublines to irradiation, highlighting DNA repair capacity as the key determinant of radiation response in our model system. Clinically, the extracted IFN/inflammation signature turned out to be a significant prognosticator for progression-free survival (PFS). In a cohort of 131 HPV-negative HNSCC cases, patients with low IFN/inflammation scores had significantly impaired progression-free-survival compared to those with high IFN/inflammation scores. Hence, expression of IFN/inflammation motifs could serve as a proxy for DNA damage repair proficiency and guide combination treatment strategies, such as ATM inhibition.
Conclusions Our findings suggest that elevated basal IFN signaling in HNSCC marks an inflamed state with impaired DNA repair and heightened radiosensitivity, while low IFN signaling correlates with greater repair proficiency and radioresistance. This study provides a framework for investigating intra-tumor heterogeneity and causal links between signaling and radiation response, with broader applications for uncovering resistance mechanisms and guiding precision therapy.

A-O2

Inhibition of polymerase theta overcomes replication-associated radioresistance in triple-negative breast cancer cell lines

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Radiation Oncology 2025, **20(s1)**:A-O2

Background Despite an initial response to therapy, triple-negative breast cancer (TNBC) tends to develop therapy resistances. This is partially attributed to activity of complex DNA repair mechanisms that protect DNA replication. Error-free "classical" DNA repair processes act alongside numerous error-prone backup mechanisms to repair DNA double-strand breaks and thus maintain the replication process. Aim of this study was to evaluate the relevance of these error-prone processes for the radiation response of human TNBC cell models in order to develop new radiotherapeutic interventions to overcome resistance.
Materials and methods A siRNA screen was conducted to analyze genes involved in replication-associated DNA damage response. Based on the colony forming ability of MDA-MB-231 cells in 3D following 6 Gy irradiation. Verification in additional TNBC cell lines and using the polymerase theta inhibitor ART558. Determination of DNA damage by the detection of γH2AX, RAD51, or RPA foci. Analysis of cell cycle by FACS and replication processes using the DNA fiber assay.
Results Significantly reduced cellular survival of up to 35% was observed for 19 of the 44 genes analyzed after siRNA knockdown and 6 Gy irradiation. Most of the top targets were associated with theta-mediated end joining (TMEJ) or translesion synthesis (TLS), both error-prone signaling pathways crucial for increased tolerance to replication stress. Among these top targets was polymerase theta (Polθ; $p < 0.01$), which is actively involved in both, TMEJ and TLS. Concordantly, siRNA knockdown and application of the clinically relevant inhibitor ART558 demonstrated radiosensitization, with an SER_{8Gy} of 1.3, ($p < 0.05$) and an SER_{8Gy} of 1.5, ($p < 0.0001$) in the 3D colony formation assay. However, significant radiosensitization was only observed at higher doses. This could be attributed to increased DNA damage ($p < 0.001$) upon Polθ inhibition and 8 Gy irradiation. Possible underlying mechanisms for the radiosensitizing effect could be the exclusive damage of S phase cells. Confirming this, increased replication stress was observed by an elevated number of RAD51 foci ($p < 0.001$) and increased S phase arrest ($p < 0.05$). And replication stress was reflected by an extension of newly synthesized DNA after Polθ inhibition alone ($p < 0.01$), but a significant slowdown after combined with irradiation. Current studies focus on the occurrence of DNA double-strand breaks and single-stranded DNA at active replication forks, both indicators of perturbations.
Conclusions Inhibition of Polθ resulted in significant radiosensitization due to enhanced DNA damage and DNA replication stress in human TNBC cell models. Although the effect of Polθ inhibition on tumor control is currently being investigated in several clinical trials, a deeper understanding of the underlying mechanisms is required for a specific targeting in combination with radiotherapy.

A-O3

Analysis of radiation-induced senescence in vitro

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Radiation Oncology 2025, **20(s1)**:A-O3

Background Radiation-induced cellular senescence is a common phenomenon in both tumor and normal tissue following radiotherapy. Changes in the cell cycle, extensive metabolic reprogramming, activation of anti-apoptotic signaling pathways, and the development of the senescence-associated secretory phenotype play a central role in both the emergence of therapy resistance in tumors and radiation-induced complications in normal tissue. Detailed mechanistic investigations are therefore urgently needed but are hindered by existing methodological limitations. This project focuses on the methodological detection of senescent cells—an ongoing major technical challenge according to current knowledge.
Materials and methods In the present study, radiation-induced senescence was examined in cell culture models of tumor tissue (breast cancer) and normal tissue (fibroblasts, endothelial cells, epithelial cells). Both qualitative-descriptive methods, such as time-lapse microscopy, immunohistochemistry, immunofluorescence, and Western blot analyses, as well as quantitative approaches, particularly FACS analyses of various properties of senescent cells, were employed. These methods were also specifically refined to enable a more robust characterization of radiation-induced senescence.
Results Published phenotypic and molecular changes associated with radiation-induced senescence exhibited a high degree of variability across the tested cell culture models and are therefore not universally applicable to all models. In particular, the detection of senescence-associated β-galactosidase showed methodological limitations, although these could largely be compensated for by combining the assay with vital dyes. Nevertheless, there remains a significant need for novel and improved markers to facilitate systematic and/or high-throughput-compatible analyses of radiation-induced senescence. To address this, transcriptomic analyses of FACS-sorted senescent cells are currently being conducted.
Conclusions In conclusion, the detection of radiation-induced senescent cells is fundamentally feasible using various methods, despite the known methodological limitations. However, improved markers are urgently required for systematic and/or high-throughput analyses.
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A-O4

Inhibition of PARP results in highly effective radiosensitization of paediatric brain tumours without affecting normal tissue

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Radiation Oncology 2025, **20(s1)**:A-O4

Background Medulloblastoma (MB) is the most and ependymoma (EPN) the third most common high-grade childhood brain tumour. Different molecular subgroups (WNT, SHH, GR3/4) of both are associated with different prognoses. Multimodal treatment leads to severe (neurocognitive) late effects. Radiosensitization approaches are of great interest as new therapeutic option to improve survival in high-risk groups and ideally to allow dose de-escalation of radiotherapy in standard-risk patients to improve neurocognitive outcome. This study investigated whether PARP inhibition (PARPi) effectively impairs DNA repair and consequently increases the cellular radiosensitivity of pediatric brain tumors. Additionally, the effect of combined treatment on non-cancerous cells was analyzed.
Materials and methods PARP expression was investigated by western blot analysis and immunofluorescence (IF). Effect of PARPi on radiosensitivity was assessed by colony formation assays. DNA double-strand break (DSB) repair capacity was analyzed in MB cell lines, astrocytes and brain tissue of healthy mice, as well as in forebrain organoids (FBO) based on human induced pluripotent stem cells and ex vivo MB and EPN slice cultures. DSBs were quantified after treatment with PARPi prior to irradiation using IF. Flow cytometric analysis was used to analyze the effect of irradiation and PARPi on cell cycle distribution.
Results GR3 MB cell lines expressed higher levels of PARP1 than SHH MB cell lines. PARPi in combination with irradiation increased the number of S/G2 phase cells and led to higher levels of residual DSBs in MB cell lines, whereas astrocytes did not exhibit significantly higher numbers of residual DNA DSBs compared to irradiation alone. MB cell lines showed increased cellular radiosensitivity when treated with PARPi prior to irradiation. PARPi in combination with irradiation resulted in decreased DNA DSB repair capacity in ex vivo tumour slice cultures of MB and EPN. Pamiparib resulted in higher numbers of DSBs than olaparib in ex vivo slices of both entities. Evaluation of the DNA DSB repair capacity in brain tissue of

healthy mice, as well as initial observations in FBOs revealed no additional DNA damage of PARPi in combination with irradiation. Proliferation, as measured by Ki67 staining, was higher in FBOs following combined PARPi and irradiation compared to irradiation alone, suggesting that PARPi may exert a neuroprotective effect by promoting cellular proliferation after irradiation.

Conclusions PARPi resulted in impaired DSB repair and enhanced radiation-induced cytotoxicity. Thus, olaparib and pamiparib are potential radiosensitizing agents for the treatment of paediatric brain tumours, which do not lead to additional damage of normal tissue.

Trial registration and/or ethics approval number All patients provided informed consent for the use of their excised specimens for research purposes. All experiments were approved by the Ethics Committee of the Hamburg Chamber of Physicians ('Hamburger Ärztekammer').

c) Proffered papers—Poster presentation

A-P1

Induction of DNA damage by far-UVC radiation in sensitive human skin

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Radiation Oncology 2025, **20(s1)**:A-P1

Background The application of far-UVC (222 nm) is being discussed as a potentially safer alternative to conventional UVC (254 nm) due to its germicidal properties. During the SARS-CoV-2 pandemic, the use of far-UVC radiation for air disinfection increasingly came into focus as a potential solution for use in public spaces, even in the presence of people. However, further research is needed, particularly for sensitive or vulnerable populations, to assess potential risks.

Materials and methods In this study, for the first time, pediatric skin (< 19 months; foreskin) and aged skin (> 60 years) were analysed under standardized conditions regarding the induction and localization of DNA damage (cyclobutane pyrimidine dimers, CPDs) following irradiation with far-UVC222 and UVC254.

The skin samples were irradiated with 30, 300, 1000, and 2000 J/m^2 of far-UVC222 and UVC254. CPD induction was analysed immediately after irradiation using fluorescence immunohistochemical staining, microscopy, and quantification of CPD-positive nuclei in a layer-specific manner (basal and suprabasal layers of the epidermis). HaCaT keratinocytes were irradiated with 10, 30, and 50 J/m^2 far-UVC222 and 30 J/m^2 UVC254. HaCaT cells were stained for CPDs and γH2AX and analysed using immunofluorescence microscopy.

Results The results clearly show that both UVC wavelengths induce DNA damage. Far-UVC222 causes significantly fewer CPDs compared to UVC254. Notably, in the basal cell layer, which is relevant for carcinogenic processes, no UV-induced CPD formation was observed after Far-UVC222 exposure, in contrast to UVC254.

At the same time, findings suggest that epidermal characteristics, such as stratum corneum and epidermal thickness, influence the sensitivity of different skin types. Additionally, the DNA-damaging effects of far-UVC through CPD and γH2AX induction are supported by investigations in cell culture.

Conclusions These results highlight the importance of wavelength filtering and the targeted consideration of individual skin characteristics to further minimize potential risks, particularly for vulnerable groups such as children and the elderly. This project focuses on the mechanistic examination of DNA damage localization and provides a basis for future safety assessments of far-UVC.

Trial registration and/or ethics approval number All cells were isolated from human foreskins derived from routine circumcisions. Obtaining ethics approval was not required. The legal guardians of the children gave informed consent before donation of foreskin samples.

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A-P2

Acute and chronic skin damage after high-dose radiation in relation to histone variant H2A.J

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Radiation Oncology 2025, **20(s1)**:A-P2

Background Radiation-related skin injuries can represent significant complications of radiotherapy, ranging in severity from transient erythema to non-healing ulcers. In addition to acute effects, chronic radiation damage includes skin atrophy with loss of hair follicles and chronic fibrosis. In earlier studies, we demonstrated that ionizing radiation prompts the incorporation of the histone variant H2A.J into the nuclear chromatin of epidermal keratinocytes, where it plays a key role in shaping acute radiation responses by modulating the expression of pro-inflammatory mediators (1; 2). To further explore the pathophysiological relevance of H2A.J in both acute and chronic radiation-induced skin damage, we conducted a comparative analysis of irradiated skin from H2A.J knockout (KO) mice and their wild-type (WT) counterparts.

Materials and methods After CT-guided planning, the dorsal skin folds of both H2A.J KO and WT mice were exposed to a single dose of 20 Gy. The skin responses to radiation were assessed macro- and microscopically for up to six months post-irradiation. The expression of H2A.J, along with important markers of cellular senescence, DNA damage, and proliferation, was evaluated using immunohistochemistry and immunofluorescence microscopy. Structural alterations within the skin layers were quantified through automated image analysis, enabling high-throughput analysis of histological changes across experimental groups.

Results As early as one week post-irradiation, WT skin exhibited marked epidermal thickening, showing a reactive proliferative response to radiation-induced injury. Interestingly, while the epidermis of H2A.J KO mice also showed significantly increased thickening, this response was delayed, peaking at two weeks post-irradiation. Following the resolution of the acute inflammatory phase, epidermal proliferation plateaued, with no further increase observed by one month post-irradiation. Three months post-irradiation, the epidermal thickness in both KO and WT mice returned to baseline levels comparable to those of non-irradiated controls.

Conclusions Radiation-induced DNA damage triggers apoptosis and cellular senescence in the epidermis, with the ensuing senescence-associated inflammatory response potentially impairing tissue repair and promoting chronic fibrotic changes. In H2A.J KO skin, this reactive proliferation is noticeably delayed compared to WT skin, yet more pronounced. These observations underline the critical role of H2A.J in shaping the skin's response to radiation injury, likely through its influence on the induction of cellular senescence and the regulation of inflammatory gene expression. The significance of H2A.J with regard to chronic fibrosis will be investigated in future studies.

Trial registration and/or ethics approval number Animal testing license (TVA 27-2021).

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A-P3

A radon biobank for the future: Advancing research on radiation-induced molecular health impacts

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Radiation Oncology 2025, **20(s1)**:A-P3

Background Radon, a radioactive gas emanating in the ground from uranium-238 decay, accumulates in indoor air and is a known risk factor for lung cancer. This has been proven by large epidemiological studies. Further biological effects as well as age- and sex-specific molecular mechanisms related to radon exposure are less well studied. Radon and its decay products release high energetic alpha particles that cause direct DNA damage and oxidative stress. Except DNA damage in directly targeted cells, effects in chronically low exposed humans remain largely unexplored. A high-quality biobank is essential to address these knowledge gaps.
Materials and methods In a nationwide survey, radon indoor concentration, was measured in more than 7,400 dwellings in two rooms for usually 1 year in Germany. Based on this data, households with high and low exposure were selected, and biological samples are being collected. Blood, blood plasma, saliva, sputum, nasal- and oral mucosal swaps are being sampled from 600 individuals (adults and minors) from households with high (> 300 Bq/m^3) and low (< 40 Bq/m^3) radon exposures. Questionnaires will gather data on time and activity spent in dwellings, health status, lifestyle and other occupational or medical exposures. Specimen are collected from people at home and sent to BfS for further preparation. Samples will be prepared for various analyses (including gamma-H2AX and chromosomal studies) and cryopreserved at the Federal Office for Radiation Protection (BfS).
Results Informed consent, study information, questionnaire and standardized operating procedures have been set up. Ethical and data protection approvals have been obtained. 407 high-exposure and 2,513 low-exposure households have been identified and contacted. 111 high-exposure households have already agreed to participate (January 2025). Low exposed participants will be matched by age and smoking status. Sample collection has started. Project completion is anticipated by 2026.
Conclusions The radon biobank, which is currently being established, will provide a diverse sample repository enabling novel research approaches, from genomic studies to analyses of the transcriptome, epigenome, microbiome, and proteome, particularly inflammatory cytokines in groups with contrasting radon exposure. The biobank will be accessible to external research groups, fostering collaborative efforts in radon-related health research.
Acknowledgements Special thanks to Ulrike Goedecke from the UMG who is responsible for collecting the samples, Juliane Pätzold, Saskia Pautz, Felix Kästle and Sven Draheim for preparation and management of the samples at the BfS.
Trial registration and ethics approval number DRKS0003472; 24020 (Ethik-Kommission der Bayerischen Landesärztekammer), 26/2/24 (Ethik-Kommission der Universitätsmedizin Göttingen).

A-P4

Analysis of irradiation-induced cell fate decisions in breast cancer

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Radiation Oncology 2025, **20(s1)**:A-P4

Background Radiotherapy (RT) plays a pivotal role in the multimodal treatment of breast cancer, including various irradiation regimens such as fractionated, hypofractionated and ablative approaches. Emerging evidence suggests that irradiation with higher single doses may (re)activate anti-tumor immune mechanisms. In this process, the characteristics and extent of irradiation-induced cell fate decisions play a crucial role. Here, we provide a comprehensive in-depth characterization of irradiation-induced cell death mechanisms, with a special focus on the regulated necrotic cell death forms necroptosis and pyroptosis.
Materials and methods In this study, irradiation-induced cell fate decisions were analyzed in a panel of 14 breast cancer cell lines and 2 non-malignant mammary epithelial cell lines via AnxV/PI flow cytometry after single dose irradiation with 10 Gy. To further subclassify the necrotic cell death fraction, established inhibitors were applied, and the identified cell death pathways were subsequently verified using specific stimulators for necroptosis and pyroptosis. The expression and activation of key regulator proteins were assessed through Western Blot analysis and immunofluorescence.
Results Single dose irradiation with 10 Gy predominantly induced necrotic phenotypes in our breast cancer cell line panel. The subclassification of the necrotic fraction using established inhibitors, along with the analysis of the expression levels of key regulators, revealed a prevalence of necroptotic and pyroptotic cell death forms, particularly in triple-negative breast cancer cells.
Conclusions Our results indicate that necroptosis and pyroptosis are the predominant cell fate decisions in breast cancer cells after irradiation with 10 Gy. The molecular mechanisms and the resulting immunological consequences of irradiation induced necroptosis and pyroptosis are currently under investigation.
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A-P5

Differential effects of IAP inhibition in irradiated glioblastoma cells—potential role for autocrine TNFα secretion

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Background Overexpression of inhibitors of apoptosis proteins (IAPs) is one of the mechanisms by which cancer is thought to evade apoptosis. Apoptosis is triggered by DNA damage but largely impaired in glioblastoma (GBM) partly due to the high IAP expression. Therefore, we hypothesize that IAP inhibition might release apoptotic cell death and synergize with irradiation (IR). Here, we investigate the impact of the IAP inhibitor xevinapant (XP) combined with IR on IAP expression, cell death and intrinsic TNFα activity in GBM cells.
Materials and methods Primary GBM cells from three different patients, p53-mutated (p53-m): P0306 and p53-wildtype (p53-wt): P0297) and four established cell lines (p53-m: T98G, LN405; p53-wt: A172, DBTRG) were analyzed. After treatment with XP (20 µM), infliximab (IFM, 10 µg/ml) and 2 h later IR (8 Gy), metabolic activity (WST-1), apoptosis (Annexin-V) and TNFα (MSD V-plex TNFα assay) were measured. Expression of IAPs (cIAP1, cIAP2, XIAP) was examined by flow cytometry.
Results In cell lines, IR led to a 1.9-fold increase of cIAP1 and 1.6-fold of XIAP fluorescence intensity. This was reduced by XP to 0.9-fold of control level but only for cIAP1 ($n = 4$, $p \leq 0.05$). Primary cells responded similarly, IR led to a higher induction of cIAP1 vs. XIAP and again, XP reduced only cIAP1 levels ($n = 2$). Basal cIAP2 level were hardly detectable but induced by IR and remained nearly unchanged by XP in all cells. Reduction of metabolic activity was pronounced by combined XP + IR treatment to 0.59 ± 0.12 compared to single treatments (XP: 0.75 ± 0.17; IR: 0.73 ± 0.08; $n = 3$; $p \leq 0.05$ vs. IR) in primary GBM cells (untreated control = 1). A cell line-dependent differential response was found in DBTRG and LN405 (XP + IR: 0.73 and 0.017). IR-induced apoptosis was detected in p53-wt (P0297) but not in p53-m (P0306) cells. However, we observed a threefold rise of apoptotic cells by combined XP + IR vs. IR treatment ($n = 2$). Similar results were found in 3 of 4 GBM cell lines, with highest response in LN405 again ($p \leq 0.05$; $n = 3$) going along with induction of TNFα secretion

(3/4) which was highest in LN405 cells. Here, inhibition of TNFα secretion with IFM reduced the percentage of apoptotic cells by 30%.
Conclusions IAP inhibition enhanced the IR-induced apoptosis in primary and permanent, p53-wt and p53-m GBM cells. Our results indicate that differential cell death responses might partly depend on autocrine TNFα secretion, warranting further investigations into its potential as predictor for IAP-inhibitory drug response.
Trial registration and/or ethics approval number Patients provided written informed consent according to German laws and in accordance with the 1964 Helsinki declaration and its amendments, as confirmed by the local ethical committee (144/08-ek).
Acknowledgements We thank Frank Gaunitz (Dep. of Neurosurgery, Leipzig) for primary cells and Anja Saalbach (Dep. of Dermatology, Leipzig) for technical support.
Conflict of interest Xevinapant was provided by Merck (CrossRef Funder ID: https://doi.org/10.13039/100009945) as part of this research project.

A-P6

Effect of UV radiation on dermal stem cell differentiation to melanocytes

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Background Malignant melanoma is the most aggressive and lethal form of skin cancer, with its main etiological factor being exposure to ultraviolet (UV) radiation. While UV-radiation is well-established as a significant cause, the exact cell of origin for melanoma remains elusive. There are two prevailing theories regarding the cells of origin of melanoma: the classical theory, which suggests that melanoma arises from fully differentiated epidermal melanocytes or melanocyte stem cells located in the bulge region of hair follicles, and the alternative theory, which proposes that multipotent dermal stem cells (DSCs), capable of differentiating into melanocytes, serve as the cellular origin of melanoma. UV-radiation, particularly UVA (320–400 nm), which penetrates deeply into the dermis, and UVB (280–320 nm), which affects the epidermis and the upper dermis, are known environmental carcinogens contributing to melanoma development. The multipotent DSCs are located in the dermis and thereby are potentially exposed to UVA and UVB-radiation. It is unclear if and how UV-radiation influences DSC differentiation into melanocytes, and what consequences pre-damaged DSCs will have for the differentiated melanocytes. This study explores the impact of UVA, UVB, and combined UVA + B irradiation on DSC differentiation into melanocytes. To this end, it is being investigated whether DSCs completely repair UV-induced DNA damage (cyclobutane pyrimidine dimers, CPD) before differentiation is initiated, or whether CPDs are still detectable during the differentiation process and in melanocytes.
Materials and methods DSCs were isolated from human foreskin tissue, cultured in stem cell medium and purified by using MACS® immunomagnetic cell sorting with NGFRp75 labeling, achieving stem cell frequencies exceeding 95%. Highly pure DSCs were cultured to 80–90% confluency and exposed to different single and multiple UV radiations After 24 h, cells were transferred to melanocyte differentiation medium and cultured for 12 days with medium changes every 2–3 days. Melanocyte differentiation was evaluated through immunostaining and quantitative PCR (qPCR) for melanocytic markers like MITF, TRP1, and HMB45. CPDs are detected with immunofluorescent staining at various times during the differentiation process. The analysis is carried out using a confocal laser scanning microscope (Leica Stellaris 5).
Results Single UVA, UVB, and UVA + B irradiation of DSCs induced morphological changes while not altering the expression of melanocyte-specific markers. These findings suggest that UV exposure do not alter the overall differentiation outcomes when compared to non-irradiated cells. Detailed analysis of differentiation state dependent CPD repair as well as the effects of multiple UV irradiations are under investigation.
Conclusions The data on repair of UV-induced DNA-damage (CPD) in dependence of differentiation state of the cell (from DSCs up to melanocytes) will give important information on the cell of origin of melanoma and thus will help to elucidate the mechanisms of melanoma development.
Trial registration and/or ethics approval number All cells were isolated from human foreskins derived from routine circumcisions. Obtaining ethics approval was not required. The legal guardians of the children gave informed consent before donation of foreskin samples.
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A-P7

Induction, complexity, and repair of neutron-induced DNA damage in peripheral blood lymphocytes

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Background Neutrons pose an increased risk of health consequences in various exposure scenarios due to their energy-dependent increased relative biological effectiveness (RBE), including in radiotherapy, manned space flight, nuclear environments, and radiation accidents. Despite extensive historical cytogenetic data, the induction, complexity, and repair of neutron-induced DNA damage has been virtually unstudied at the molecular level.
Materials and methods Venous whole blood samples from 2 healthy donors were irradiated with 140 kV X-rays (dose range: 0–4 Gy), monoenergetic neutrons (556 keV and 1.2 MeV, dose range 0–100 mGy and 0–200 mGy, respectively) or a neutron fission spectrum (0.1–8 MeV, dose range 0–1 Gy). Samples were processed for quantification of DNA double-strand breaks (DSBs) as foci of surrogate markers γH2AX, 53BP1, and phospho-RPA 2 h and 24 h after irradiation in G1 lymphocytes, the cytokinesis block micronucleus (CBMN) assay and unstable (Giemsa staining) as well as transmissible and complex (mFISH staining) chromosomal aberrations in first post-exposure metaphases and G2 prematurely condensed chromosomes.
Results The CBMN assay confirmed an increased mean neutron RBE based on the linear regression coefficient of the dose–response relationships, which was 7.4 for 556 keV, 6.1 for 1.2 MeV, and 6.0 for the fission spectrum. However, the dose-dependent linear induction of colocalizing γH2AX and 53BP1 foci per cell 2 h after irradiation was comparable between X-rays (10.3 foci/Gy) and 556 keV (10.1 foci/Gy) or 1.2 MeV (10.3 foci/Gy) neutrons. We were able to demonstrate complex DSBs and their resection-dependent repair by successfully detecting colocalizing γH2AX and phospho-RPA foci in G1 lymphocytes 2 h after fission spectrum exposure. Preliminary mFISH screenings confirmed the high and complex burden of chromosomal aberrations due to neutron exposure, which we will examine in detail.
Conclusions Our data confirm the increased RBE of neutrons at various energies for cytogenetic effects. However, demonstrating the differential impact of radiation qualities on the complexity of DSBs required resection-dependent repair markers, which can be implemented for biodosimetric purposes after radiation accidents. The transmissibility and complexity of neutron-induced chromosomal damage will shed light on the health consequences including carcinogenic risks.
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Trial registration and/or ethics approval number Ethical approval was obtained from the Medical Association of Rhineland-Palatinate [No. 2023-17191].

A-P8

Impact of UV radiation on cell cycle regulation, apoptosis, and epigenetic modifications in dermal stem cells

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Background The incidence of melanoma increased over the past decades. Although it accounts only 1% of skin cancers, melanoma is responsible for more than 80% of skin cancer mortality. The etiology of malignant melanoma (MM) is very complex and involves both environmental and genetic factors. Epidemiological studies have identified UV radiation as the main risk factor for the development of MM, especially recurrent, intense sun exposure. Both ultraviolet A (UVA) radiation (320 to 400 nm) and ultraviolet B (UVB) radiation (290 to 320 nm) contribute to the development of melanoma. It has been suggested that differentiated epidermal melanocytes accumulate UV-induced mutations in oncogenes and tumor suppressor genes and progressively degenerate to metastatic MM. Recently, melanocytic stem cells (MSCs) have been identified as potential target cells for the carcinogenic effect of UV radiation. Besides MSCs in bulge region of hair follicles, neural crest-derived dermal stem cells (DSCs) represent a reservoir for melanocytes. UVA can penetrate into the dermis and UVB to some extend into the upper layers of the dermis and therefore has the potential to damage stem cells localized there. It is assumed that during differentiation to melanocytes, DSCs migrate from the dermis to the epidermis and are subsequently exposed to increasing doses of UVA and UVB.
Stem cells differ from differentiated cells in several aspects such as cell cycle distribution, gene expression, chromatin organization, epigenetic pattern, and DNA damage response. Increased apoptosis rate in damaged stem cells is a well-known phenomenon as a protective mechanism against carcinogenesis. Therefore, when considering MM genesis, it is crucial to understand how DSCs cope with UV exposure.
Materials and methods Human primary DSCs are isolated from juvenile foreskin. UV exposure is performed as single and multiple irradiations for UVA, UVB and their combination. The effects of UV exposure for DSCs compared to fibroblasts and melanocytes from the same donors are analyzed by flow cytometry for assessment of cell cycle and apoptosis and by western blot for checkpoint protein analysis. Histone modification changes are detected by immunofluorescence.
Results Results for cell cycle perturbations in DSCs, fibroblasts and melanocytes as well as radiation sensitivity will be presented.
Conclusions Understanding the UV damage response in dermal stem cells is essential for elucidating melanoma initiation and progression.
Trial registration and/or ethics approval number All cells were isolated from human foreskins derived from routine circumcisions. Obtaining ethics approval was not required. The legal guardians of the children gave informed consent before donation of foreskin samples.
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A-P9

Incorporating cooperative and competitive interactions in clonogenic growth analysis in vitro

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Background The colony formation assay (CFA) and the limiting dilution assay (LDA) are two widely used methods to quantify self-renewal capacity and clonogenic cell survival in vitro. Both methods measure the ability of single cells to form colonies, CFA directly through the number of colonies grown from given numbers of single cells, LDA indirectly through the proportion of successful growth events in setups with diluted cell numbers. Both assays offer essential insights into clonogenic potential but are fundamentally based on the assumption that individual cells act independently. This assumption is frequently violated due to cooperative or competitive interactions, introducing bias and high uncertainty into clonogenicity measurements.
Materials and methods By generalizing the mathematical model for clonogenicity quantification from the special case of non-interacting single cells to power-law relationships between cell numbers and growth success rates, we relax the strict statistical assumption of cellular independence. This allows for a more accurate fit of experimental observations influenced by cooperative or competitive interactions and thus enables a more comprehensive determination of the effective clonogenicity. The derived mathematical equations are analyzed for uncertainties and error propagation.
Results Due to the nature of mathematical generalizations, the non-cooperative and non-competitive case remains unchanged and is seamlessly integrated into the updated versions of both analyses. For all other scenarios, the updated equations provide more robust and reliable clonogenicity measurements and are made accessible through the R packages CFAcoop and LDAcoop, as well as online tools (or an Excel tool in the case of CFAcoop). These tools enable users to apply the generalized models without requiring advanced statistical or programming expertise.
Conclusions Disregarding possible deviations from the assumption of independent cell behavior introduces systematic biases in the measurement of clonogenic potential in mammalian cells and, consequently, in the analysis of survival fractions after treatment. The use of the provided tools, CFAcoop and LDAcoop, enables robust and reliable quantification of clonogenic potential while accounting for cooperative and competitive effects in the clonogenic growth of cell populations.
Funding This work was supported by the Deutsche Forschungsgemeinschaft DFG (SFB1321, Project-ID 329628492, P16), the Bundesministerium fuer Bildung und Forschung BMBF (ZiSStrans NUK047A, NUK047C, METABOLiST 02NUK061A, NUK061C, SeniRad 02NUK086A and DKTK) and the International graduate program iTarget (Elitenetzwerk Bayern).

A-P10

Topological dynamics of the nano-organization of chromatin and DNA repair foci in relation to the radiosensitivity of human cell lines

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Background Ionizing radiation is used in radiotherapy to treat various types of cancer. In order to study DNA repair different cancer and non-cancer (control) cell lines can be used. DNA double strand breaks (DSB), induce the formation of γH2AX foci followed by attaching and detaching of repair proteins. The cell nucleus, however, is a complex system in which simultaneous reactions and functions take place to keep the cell well-running. Between chromatin, there is space for trafficking of the repair proteins. In order to improve the accessibility of the damage sites the whole system "cell nucleus" responds by chromatin reorganization.
Materials and methods In this study, super-resolution Single Molecule Localization Microscopy (SMLM) was applied in combination with mathematical analyses of point patterns (Ripley statistics, persistent homology, persistent imaging and principal component analysis (PCA)) of the molecular label coordinates obtained [1]. These geometric and topological analyses were used to investigate the chromatin dynamics during repair processes and to correlate the outcome with radio-sensitivity. Spatial structures of γH2AX and 53BP1 labelling sites were analyzed in relation to their repair mechanisms. Nanoscale differences of chromatin and repair foci organization between cancer and non-cancer cells may explain basic mechanisms of repair processes.

Results PCA on the whole chromatin topology showed a high variance for all cell lines, which was cell type-specific [2]. The results showed that DNA damage responses were embedded into a response of the whole chromatin system [3,4]. The whole chromatin topology revealed a cyclic movement in the latent space of the two major principal components during a successful repair of DNA-DSBs non-cancer cells. This means that after repair the chromatin topology reached the non-irradiated control again [1]. In contrast, a successful repair outcome in cancer cells could reveal a non-cyclic outcome, i.e. the chromatin topology was different after repair from the non-irradiated cells. The topology of yH2AX and 53BP1 clusters also revealed repair time depending cyclic course in the latent space for the different cell lines depending on the radiation and treatment applied.
Conclusions Principal component analysis proved to be a powerful tool to determine basic topological features. The results support the hypothesis that chromatin organization correlates with radio-sensitivity and impacts the control of repair.
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A-P11

Histone variant H2A.J is essential for the maintenance of adult neurogenesis, particularly following exposure to ionizing radiation

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Background The hippocampal formation, along with various cortical regions, is vital for short-term memory and learning. A process known as adult neurogenesis—primarily occurring in the dentate gyrus—is essential for these cognitive functions [1]. Our previous research demonstrated that the histone variant H2A.J is incorporated into nuclear chromatin through epigenetic mechanisms following ionizing radiation (IR), influencing gene expression and cellular activities associated with radiation-induced senescence. In this study, we aimed to explore the pathophysiological role of H2A.J in the brain's response to IR [2].
Materials and Methods Wild-type (WT) and knockout (KO) mice were subjected to either a single dose of 10 Gy IR or fractionated IR consisting of 5 doses of 2 Gy each. 24 and 72 h post-irradiation, animals were anesthetized, perfused, and their brain tissues collected. Brain sections from both irradiated and non-irradiated age-matched controls were stained for markers such as DCX, SOX2, GFAP, and OLIG2. Automated image analysis was used to quantify different cell populations within the hippocampus.
Results Neurogenesis was markedly diminished in brains of non-irradiated KO mice, evidenced by scarcity of DCX + and SOX2 + neuroprogenitors. Following IR exposure in WT mice, there was a substantial decline in neurogenesis accompanied by reactive increase in GFAP + astrocytes and OLIG2 + oligodendrocytes within the hippocampus; most glial cells exhibited radiation-induced H2A.J expression. Conversely, KO mice did not show a similar glial cell increase after IR.
Conclusions The absence of H2A.J in KO brains results in significantly reduced neurogenesis even without IR exposure. Additionally, KO brains seem to have a diminished capacity to respond effectively to radiation-induced damage in the hippocampus. These findings suggest that H2A.J plays a critical epigenetic role in maintaining neurogenesis and facilitating regeneration following radiation injury within the hippocampal niche.
Trial registration and/or ethics approval number Animal experiment licence TVA 29-2020.

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Session B: Signaling and adaptive mechanisms in cellular responses to radiation

a) Key note lecture

B-K1

Experimental data from FLASH—Which factors matter

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Preclinical studies have demonstrated that ultra-high dose rate radiation spares normal tissue, while tumor responses have thus far remained equivalent to those observed with conventional dose rates. This phenomenon is referred to as the FLASH effect. This differential response holds promise for improved clinical outcomes; however, significant uncertainties persist regarding the underlying biological mechanisms of FLASH radiotherapy.
Current evidence suggests that multiple factors may modulate the extent of tissue sparing, including dose rate, temporal irradiation pattern, tissue type, total dose, and fractionation schedule. We have systematically investigated the FLASH effect through a series of in vivo studies employing both proton and electron FLASH modalities, utilizing a well-established murine leg model at the Danish Centre for Particle Therapy [1–4]. These investigations have encompassed the influence of dose rates, temporal structure, fractionation, and oxygenation levels within the irradiated tissue. By concurrently assessing two normal tissue endpoints, acute skin toxicity and radiation-induced fibrosis, in the same animals, we enabled a direct comparison of the endpoint-specific manifestations of the FLASH effect.
Our findings indicate that the magnitude of the FLASH effect is influenced by both dose rate and fractionation, with notable differences observed between acute and late tissue responses. Furthermore, the detected FLASH effect is contingent upon the dose sensitivity of the assay employed, highlighting that conclusions drawn from single-dose experiments, such as the determination of a dose rate threshold for FLASH, are inherently dependent on the sensitivity of the analytical method utilized [5].
Acknowledgements Some of the included studies are financially supported by Varian**.**

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b) Proffered papers—Oral presentation

B-O1

Hypofractionated particle therapy improves tumor control and preserves healthy tissue in brain tumor organoids

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Background Hypofractionation is often used in C-ion radiotherapy thanks to the high precision of the heavy ion beams. However, reducing the number of fractions can lead to increased normal tissue toxicity. We compared the toxicity and tumor cell killing of 1 fraction (15 Gy) vs. 3 fractions (3 × 7.5 Gy) with an equivalent biologically effective dose (BED) for tumor ($\alpha/\beta = 10$ Gy) using human cerebral organoids as brain cancer model.
Materials and methods Normal brain organoid slices generated using human pluripotent stem cells and cultured alone or with genetically modified brain tumor-like cells ($GFP^+/cMYC^{high}$ and/or $p53^{-/-}/NF^{-/-}/PTEN^{-/-}$) in an autologous setting were irradiated with high-energy C-ions and assessed for cell death, gene, and protein expression at multiple time points (7, 20, and 70 days) post-irradiation.
Results A single 15 Gy dose induced a transient increase in extracellular lactate dehydrogenase (LDH), a marker of necrosis, which was absent after 3 × 7.5 Gy exposure. No significant changes in LDH levels were observed at later time points in normal organoid slices. Tumor cell killing was demonstrated by reduced GFP^+ cells and decreased cMYC mRNA expression. At day 70 post-irradiation, regrowth of tumor-like cells was observed after the single dose but not in the hypofractionated regimen. Markers for astrocytes (GFAP) and neurons (MAP2) were largely unaffected in normal organoid slices but increased in co-cultures after irradiation, especially after single-dose exposure, compared to sham-irradiated controls with low levels of these markers, attributed to the predominance of tumor-like cells. The surrounding normal organoid astrocytic cells influenced the radiosensitivity of GBM-like cells, their survival, and invasive potential resembling tumor aggressiveness in the recurrence. No significant changes in necroptosis marker MLKL were observed after irradiation of normal slices, although MLKL expression decreased over time in co-cultures.
Conclusions Hypofractionated C-ion radiotherapy results in reduced normal tissue toxicity and more efficient inhibition of tumor cell regrowth in brain organoids at later time points compared to a single high dose at the same BED. These findings suggest that hypofractionation may provide a more favorable balance between therapeutic efficacy and normal tissue preservation, particularly in the treatment of brain tumors. Our results also underline the importance of cell–cell interactions between normal and tumor tissue in response to GBM therapies.
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Trial registration and/or ethics approval number Experiments were conducted using the feeder-free human embryonic stem cell line WA09-FI (H9), which was utilized in accordance with §4 and §6 of the German Stem Cell Act (registry numbers 3.04.02/0125 and 3.04.02/0125-E01).

B-O2

Spatial astrocyte distribution in mice after partial-brain proton irradiation

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Background In recent years, several clinical studies reported unexpected radiation-induced side effects in healthy brain tissue after proton therapy [1]. There is speculation on the role of blood–brain barrier (BBB) disruption promoting the development of side effects [2], with astrocytes being an integral functional element of the BBB. To investigate the brain's response to proton radiation, a preclinical mouse model was established at the University Proton Therapy Dresden [3]. Based on data from this experiment, the aim was to evaluate the impact of dose on the spatial distribution of astrocytes within the brain.
Materials and methods Mice were irradiated with collimated (4 mm) proton beams, targeting the right hippocampus and stopping in the right hemisphere. Different dose levels were considered with a single fraction of 40–85 Gy or 0 Gy control. Mice were sacrificed after six months, brains were excised, and the whole brain was axially cut and histologically stained against eight markers. Here, a pipeline was established to analyze brain slices that were stained by glial acidic fibrillary protein (GFAP), which targets astrocytes. A pilot study considers single mid-brain slices including the hippocampus from an intermediate dose group (65 Gy) and control cohort (0 Gy). The astrocytes were segmented after background subtraction and applying Otsu threshold with ImageJ. For each segmented object, the centroid coordinates were calculated and counted within tiles of 100 µm × 100 µm. For statistical analysis, a rectangular brain sub-region was defined in the right hemisphere, which was centered along the beam axis and contained the dose maximum, and was mirrored in the left hemisphere, which was not irradiated.
Results Comparison of the targeted right and the left hemisphere showed a clear pattern of increased numbers of astrocytes on the irradiated side, roughly following the shape of the dose distribution. In contrast, the astrocyte distribution within the 0 Gy control was highly symmetrical, mainly following anatomical boundaries between brain regions. The number of astrocytes in the evaluated rectangular subregions was significantly higher for irradiated compared to non-irradiated mice. However, for both irradiated and non-irradiated regions, the cerebral cortex showed lower astrocyte counts.
Conclusions Histological slices of mouse brains previously irradiated with protons at the right hippocampus showed a clear dose-dependent distribution of astrocytes with significantly increased numbers in high-dose regions. Combined with analyses of other markers available in the dataset, further insight into the role of the BBB in modulating radiation-induced side effects is expected.

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B-O3

Damage-specific p21 response to radiation and toxin exposure

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Background The cyclin-dependent kinase inhibitor p21 is at the center of the DNA damage response (DDR). It is activated by a wide variety of genotoxic events, and promotes cell cycle arrest by inhibiting the activity of cyclin-dependent kinases, the key regulators of cell cycle progression. While this canonical function is well established, it remains questionable whether all cells in a culture or tissue respond in a uniform manner. In addition, the cell type and the dose and type of genotoxic stress may also determine how DDR target genes such as p21 are affected, thereby defining the nature and extent of the cellular response to a specific toxin. Here, we set out to determine radiation- and toxin-specific kinetics of p21 expression and, further downstream, cellular consequences of its induction.
Materials and methods To monitor the individual p21 expression of cells in a given culture of two different cell lines, we tagged the endogenous CDKN1A gene of HT-1080 (cancerous) and MCF-10A (benign) cells by fusing the GFP sequence to it via CRISPR/Cas9 editing. We determined by flow cytometry the dose–response relationship of p21 induction to different radiation sources (X-rays, UVA, UVB) and genotoxic chemotherapeutics (camptothecin, etoposide). By the same approach, we determined the kinetics of γH2AX induction by these damages. We also generated by CRISPR/Cas9 editing p21-knockout clones of both cell lines to uncover by next generation sequencing (NGS) of their transcriptome potential p21-specific cellular responses to radiation and genotoxins.
Results We find, in both cell lines, that X-rays, UVA and UVB radiation, and the two anti-tumorigenic topoisomerase inhibitors camptothecin and etoposide each have characteristic dose–response curves which are similar to those of γH2AX induction. X-rays cause strictly linear dose-dependent increases in p21-GFP expression, whereas UVA and UVB evoke sigmoidal dose response curves. Both chemotherapeutics, however, yield Michaels-Menton-like kinetics. In addition, first findings of our NGS approach will be presented.
Conclusions Our findings indicate that p21-GFP-edited cell lines represent a universal cellular biomarker for highly diverse toxic stimuli. They also show for the first time that p21 activation is specific to each damage type, allowing qualitative comparison of cell responses to radiation or anti-tumor drugs. Knowledge of transcriptional alterations in wild-type *versus* p21-knockout cells may teach us whether the highly specific p21-damage-response curves observed here translate to likewise damage- and p21-dependent gene expression dictating whether a cell will fully recover, senescence, or die by e.g. apoptosis.

c) Proffered papers—Poster presentation

B-P1

Identification of senescence mechanisms in HNSCC cell lines and patient cohorts

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Background Radiotherapy is an essential element in the multimodality treatment of advanced head and neck squamous cell carcinoma (HNSCC), but despite aggressive therapy, the 5-year survival rate for advanced HNSCC is only around 50%. The reasons for treatment failure are complex but are mainly attributed to intrinsic radioresistance of tumor cells. Recent publications highlight an important role of cellular senescence for radioresistant tumor phenotypes, but a deeper understanding of the molecular mechanisms of senescence in tumor cells is still lacking. Furthermore, so far, neither exclusive markers for senescence, nor universal signatures are described to identify senescent tumor cells.
Materials and methods A panel of 11 human HNSCC cell lines was systematically characterized for intrinsic radioresistance and cellular senescence after irradiation, and molecular profiling was conducted. Basal, chemical- and radiation-induced senescence was quantified, characterized at the cellular level, and single cell and bulk transcriptome profiling was performed to extract HNSCC-specific mechanisms of senescence. For validation, publicly available datasets and two well-characterized internal HNSCC patient cohorts were used.
Results There were distinct differences in senescence between individual cell lines, thus in-depth analyses were performed with selected cell lines showing pronounced phenotypes. Preliminary RNA-Seq data suggest specific transcriptomic changes associated with radiation-induced senescence.
Conclusions Recently, cellular senescence gained great attention for cancer treatment due to the availability of senomorphic and senolytic drugs for clinical application. Therefore, there is an urgent need to reliably identify senescent tumor cells. Furthermore, senescence programs of tumor cells and different cell types of non-tumorous tissue are poorly characterized so far. This study aimed at the identification of cell type-specific differences in senescence mechanisms in order to identify potential molecular targets for more efficient therapy options in HNSCC.
Trial registration and/or ethics approval number Ethical approval (EA) for this study was obtained by the ethics committee of the LMU (EA 312-12, 448-13, 17-116).
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B-P2

Investigating a potential caveolin 1-senescence-axis within radiation-induced lung injury

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Background Radiation-induced lung injury, a severe side complication following thoracic irradiations, was already linked to senescence of lung epithelial cells, which together with the developing hypersecretory phenotype (SASP) determine normal lung toxicity. At the same time, alterations in the membrane protein caveolin-1 (CAV1) are supposed to be critically involved in lung tissue damaging processes also can act as regulator of cellular senescence. However, a cell-type dependent role of CAV1 for radiotherapy (RT)-induced senescence remains to be clarified.
Materials and methods Using the preclinical mouse model of RT-induced pneumopathy together with wild-type (WT) and CAV1-deficient littermates we first investigated potential CAV1 alterations and senescence marker expressions within irradiated lungs. In parallel, the impact of RT on cellular features (cell cycle, viability, apoptosis/ cell death/ survival, senescence, colony formation) was investigated in vitro using bronchial epithelial cells, lung endothelial cells and fibroblasts in a CAV1-dependent manner in order to establish a potential CAV1-senescence-axis. Beside classical 2D monocultures, the impact of cellular crosstalk using transwell co-cultures systems of different normal lungs cells was analyzed.
Results Lung irradiations in WT mice caused a decline of overall CAV1 levels, an effect that came along with RT-induced senescence in bronchial epithelium. Up to now, induced senescence could not be detected in other cell types, e.g., endothelial cells or fibroblasts. At the moment, the impact particularly of senescent fibroblasts in fibrosis-prone CAV1-defincient animals of remains to be clarified in current investigations. RT induced senescence levels in lung epithelial and endothelial cells could not be linked to declining CAV1 levels. Declining CAV1 levels following RT were observed in fibroblasts, together with induced senescence. A reduction of CAV1 levels in fibroblasts using shRNA in turn dis not impact on the degree of senescence induction, although these cells are considered to be more radioresistant. Currently, senolytic or/and or CAV1-targeting

agents are investigated to modulate RT-induced senescence in monocultures and more complex epithelial-fibroblasts spheroidal co-cultures to gain more insights into the supposed CAV1-senescence axis and to finally unravel potential radioprotective effects.
Conclusions Declining CAV1 levels following RT and induced senescence could be observed only in lung fibroblasts. A connection between cellular CAV1 levels and senescence maybe cell type- and/or tissue-dependent. Herein, the spatiotemporal regulation of cellular CAV1 could be decisive for the onset of (RT-induced) cellular senescence.
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B-P3

Impact of RBE model and radiosensitivity parameter choice on carbon ion treatment plans for non small cell lung cancer

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Background Radiotherapy with carbon ions is associated with an increased biological effect per Gy, quantified by the Relative Biological Effectiveness (RBE). It is considered in treatment planning by the use of dedicated RBE models. Non small cell lung cancer (NSCLC) has been successfully treated with hypofractionated carbon ion therapy at the National Institute of Radiological Sciences (NIRS) in Japan, where the Microdosimetric Kinetic Model, based on radiobiological data for HSG cells, was used to predict the RBE. In contrast, in European facilities, the Local Effect Model (LEM) I is used and conventionally an $\alpha/\beta = 2$ Gy is assumed. While conversion strategies have been developed, we here investigate the impact of (i) changing to a more recent and improved model LEM IV and (ii) of adopting $\alpha/\beta = 6$ Gy which has shown to be more representative of late lung tissue response to radiation.
Materials and methods Four test geometries were selected to reflect early and locally advanced stage NSCLC at two different depths. For RBE calculation with LEM IV, $\alpha/\beta = 6$ Gy was chosen in agreement with dose response studies for NSCLC. Treatment plan optimizations were conducted with the GSI in-house treatment planning system TRiP98. Multiple optimized LEM treatment plans were inspected to find those best matching the NIRS physical target dose distributions, thereby finding dose conversion factors between MKM and LEM.
Results Prescribed doses needed for a NIRS-matched biological response were strongly dependent on the choice of model as well as the α/β ratio. The higher the number of fractions, the higher doses with LEM IV relative to those with MKM had to be selected to receive similar biological effect. For deeper or smaller targets, lower doses had to be prescribed with LEM than for bigger targets or smaller depths. These characteristics can mainly be attributed to the stronger RBE variation with depth of LEM IV due to a higher weighting of complex DNA damage predominantly caused by high LET radiation. This RBE variation is less pronounced for $\alpha/\beta = 6$ Gy as compared to 2 Gy.
Conclusions Choice of model and the α/β ratio strongly impact translation between treatment planning systems based on different RBE models. Target geometry also had an impact, although to a smaller degree. Consideration of the appropriate α/β ratio allows the derivation and investigation of biologically oriented treatment plans for lung cancer with LEM IV.

B-P4

Genome-wide transcriptomic response of whole blood after X-ray exposure

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Background The highly radioresponsive hematological system is affected in almost every exposure scenario to ionizing radiation. Whole transcriptome analyses offer a comprehensive view of radiation-induced effects in the blood, delivering valuable insights for biodosimetry in radiation accidents or immune-related responses to radiotherapy.
Materials and methods Medium diluted whole blood samples from 2 male donors and 1 female donor were exposed to 0, 0.5, 1, 2, and 4 Gy of X-rays and incubated for 2 h and 6 h at 37 °C. Whole RNA was isolated and sequenced with Illumina. Differentially expressed genes (DEGs) were extracted and considered significant at a false discovery rate (FDR) cut-off of 0.05. Functional enrichment analysis was performed using ClusterProfiler R package. Transcription factor inference activity was calculated using decoupleR package.
Results Gene expression was subject to strong inter-donor variation and time post-exposure. After 0.5, 1, 2, and 4 Gy X-rays exposure, 5, 33, 84, and 364 genes (2 h) and 72, 99, 274, and 607 genes (6 h) were differentially expressed, compared to 0 Gy. The corresponding number of the inferred significantly regulated transcription factors was 255, 253, 274, and 292 after 2 h and 214, 245, 262, and 279 after 6 h. In sham-irradiated blood, 924 DEGs and 126 transcription factors were affected by ex vivo incubation alone. Low X-Ray doses ≤ 1 Gy stimulated DNA damage response, while high doses ≥ 2 Gy triggered additional proinflammatory responses. Interestingly, we identified 34 DEGs that were not previously described as radiosensitive genes. Among these genes, 8 and 9 showing significant positive or negative correlations with dose, respectively, including GPN1, MRM2, G02S, and PTPRS.
Conclusions This first genome-wide RNA-Seq study of ex vivo X-Ray-irradiated human blood reveals novel radiosensitive genes and pathways, enhancing the understanding of the consequences of diagnostic, therapeutic, or accidental exposures on the highly radioresponsive hematological system.
Trial registration and/or ethics approval number As part of the PhyBioN project, the BMBF, Grant 02NUK084A, and the DFG, Grant 318346496 - SFB1292/2 TP19N supported this study. The study involves human participants, and the ethical approval was obtained from the Medical Association of Rhineland-Palatinate No. 2023 - 17191.

B-P5

Measuring of ROS and autofluorescence to determine senescence induction of breast carcinoma cells after irradiation

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Background Irradiation of cells can provoke different cell responses such as apoptosis, necrosis, differentiation but also senescence, depending on the dose and the cell system investigated. Previous studies have shown that irradiation of tumor-associated fibroblasts can result in the development of an inflammatory phenotype and senescence, contributing to therapy resistance and tumor progression. Reactive oxygen species (ROS) play a role in stress-induced senescence. Increased ROS concentrations and autofluorescence also correlate with senescence in tumor and normal tissue cells. The aim of the project was to establish ROS and autofluorescence measurements for the detection of radiation-induced senescence in breast carcinoma cells.
Materials and methods The breast carcinoma cell lines MCF-7, BT549 and HCC1937 were irradiated with a single dose of 8 Gy (X-rays). Cellular

autofluorescence and ROS (2',7'-dichloro- dihydrofluorescein diacetate) were measured by flow cytometry at 0, 1, 2, 4 and 7 days after irradiation assessing mean fluorescence intensities (MFI).
Results Irradiation of MCF-7 cells increased the intracellular ROS levels already on day 1 to 1400778 MFI compared to 966469 MFI in non-irradiated cells. ROS levels were significantly increased on day 2 (1404230 vs. 715364 MFI), day 4 (1232043 vs. 652459 MFI) and reached a 2.4-fold increase on day 7 (1127938 vs. 466069 MFI). BT549 cells showed a significant 1.42-fold ROS induction on day 1 after 8 Gy (565078 vs. 398469 MFI), and a 1.56-fold induction from 455344 MFI in control cells to 709126 MFI in irradiated cells. In contrast, HCC1937 cells only showed a 1.33-fold increase on day 1 (1092468 vs. 819204 MFI), while no difference between irradiated and non-irradiated cells was measurable on day 7. The detection of autofluorescence confirmed the cell line-dependent senescence induction with lower values between 3000 and 9400 MFI.
Conclusions Irradiation induces more senescence in MCF-7 cells compared to BT549 and HCC1937 cells. The induction of radiation-induced ROS levels correlates with increased autofluorescence of irradiated breast carcinoma cells. Both methods seem feasible to determine radiation-induced senescence, which will be verified in further studies on normal cells and in comparison with other measuring techniques.
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B-P6

Ex-vivo 53BP1/γH2AX-foci assay of human NSCLC tumors after irradiation with carbon ions and photons

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Background Non-small cell lung carcinoma (NSCLC) and its still poor survival prospects are a continuing challenge for radiotherapy. Therapy with carbon (^{12}C) ions enables the optimal protection of healthy lung tissue and surrounding organs. We analyze the DNA double-strand break (DSB) repair and the effect of ^{12}C-ion radiation on the PI3K/mTOR & MAPK pathways with a human ex vivo assay of lung tumor tissue.
Materials and methods Precision cut lung slices (PCLS) were excised during surgery and treated with the PI3K & mTOR inhibitor NVP-BEZ235. Following the treatment, the PCLS were irradiated with 1 Gy ^{12}C ions or 3 Gy photons and fixated after 1 and 24 h. The PCLS got sliced to 5 µm thickness with a cryotome and placed on microscopy slides. The slices were stained with immunofluorescence against the DSB marker proteins 53BP1 and γH2AX. The slides were viewed under a fluorescence microscope and the recorded images were automatically analyzed with ImageJ/FIJI software for their foci counts.
Results We generated PCLS from 5 patients. All samples showed a high induction of DSBs at 1 h after irradiation. The initial high amount of DSBs could be repaired after 24 h except for a small proportion of residual DSBs. The ongoing analysis reveals a patient/tumor-sample individual response towards irradiation. Over all patients tumors assayed, the BEZ235 samples showed a greater amount of foci compared to the irradiated DMSO control.
Conclusions The ex vivo assay is a well-suited tool to analyze active components like PI3K/mTOR inhibitors in combination with different radiation types in the human tumor environment. With this method we can show the radio-sensitizing effect of BEZ235 after ^{12}C-ion irradiation in human lung tumor tissue. If radiotherapy can be combined with sensitizing inhibitors that are administered at a low dose, then the surrounding healthy tissue can be better protected due to a possible dose reduction during therapy.
Acknowledgements This project is funded by resources of the Hessian Ministry of Science and Art (MIT-Research of the Philipps-University Marburg) and the German Federal Ministry of Education and Research (PARTITUR 02NUK076B).
Trial registration and/or ethics approval number Ethical approval for this study was obtained from ethics committee of Justus-Liebig University Gießen, faculty of Medicine (approval number 58/15).

B-P7

Transcriptomic Profiling of the UV-induced DNA Damage Response in Dermal Stem Cells

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Background Melanoma represents the most aggressive and lethal form of skin cancer, with ultraviolet (UV) radiation exposure serving as a primary etiological factor. The precise identity of the progenitor cell harboring the DNA damage necessary for melanoma initiation remains an area of active investigation. According to the traditional paradigm, melanoma originates from transformed, mature epidermal melanocytes or melanocyte stem cells residing in the bulge region of hair follicles. However, an alternative hypothesis has recently gained traction, suggesting that extrafollicular melanocyte stem cells, specifically dermal stem cells (DSCs) located within the human dermis, may function as the cell of origin. These multipotent precursors, derived from the neural crest, are of particular interest due to their dermal localization, which subjects them to UVA radiation, capable of penetrating deeper tissue layers, and UVB radiation, which predominantly affects the superficial dermis.
Materials and methods This study involved the isolation of dermal stem cells (DSCs) from juvenile human foreskin to investigate the molecular and transcriptomic responses to solar ultraviolet (UV) radiation in comparison to melanocytes. The UV-induced DNA damage response was characterized by analyzing temporal transcriptomic changes at early (6 h post-irradiation) and late (24 h post-irradiation) intervals. DSCs and melanocytes were extracted from foreskin samples (n = 5) and exposed to controlled single and repetitive UVB radiation doses. Comprehensive donor-matched transcriptomic profiling was conducted using bulk RNA sequencing on the Illumina NextSeq 1000 platform to elucidate the molecular pathways involved in UVB-induced damage and repair. Further, it was followed by spatial transcriptomic profiling to elucidate the spatial pattern of the candidate genes selected using NGS experiments.
Results Our findings reveal that dermal stem cells (DSCs) exhibit a significantly more dynamic transcriptomic response than melanocytes following irradiation showing a 5- to tenfold increase in the number of differentially expressed (DE) genes. Gene ontology (GO) biological process analysis identified that these DE genes are primarily associated with DNA repair, chromatin remodeling, and apoptosis.
Conclusions This study represents the first in-depth transcriptomic profiling of DSCs, emphasizing their stronger response to UV exposure relative to melanocytes, which may render DSCs more susceptible to UV-induced damage. These results establish DSCs as a critical model for exploring UV-driven melanomagenesis.
Trial registration and/or ethics approval number All cells were isolated from human foreskins derived from routine circumcisions. Obtaining ethics approval was not required. The legal guardians of the children gave informed consent before donation of foreskin samples.

B-P8

Impact of lamin mutations on radiation response in lung cancer

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Background Lamins are intermediate filament proteins that provide structural support to the nucleus. They form dimers that assemble into tetrameric filaments forming a meshwork of polymerized lamins. Depolymerization is induced by phosphorylation enabling cell cycle progression and cell division. Lamins also play critical roles in maintaining nuclear integrity, regulating nuclear stability. Additionally, they have been reported to impact the DNA damage response and the organization of chromatin structure [1]. It was shown that p53 stabilizes lamin A/C in response to DNA damage, while the loss of p53 is associated with an upregulation of lamin B1 expression, indicating a role for lamin regulation in cellular senescence upon DNA damage [2, 3]. Overall, changes in lamin expression are frequently observed, while lamin mutations are relatively rare in cancer. These changes can result in chromosomal and genomic instability, which are hallmarks of cancer. We hypothesize that the nuclear envelope network is linked to DNA damage response and cell cycle regulation upon irradiation in lung cancer cells and thus represents an important cell-autonomous mediator of radiosensitivity.
Materials and methods CRISPR/Cas9-mediated approaches were used to generate lamin phosphorylation mutations (unfeasible or persistent phosphorylation) in lung cancer cell lines by site-directed gRNAs and homology-directed repair. Selected single clones enable further investigation of lamin function by analyzing proliferation and subcellular localization of lamins and their mutated variants using immunofluorescence. Furthermore, this study assesses the impact of lamin mutations on nuclear stability and the DNA damage response by using γ-H2AX staining and the comet assay.
Results So far, mutations at three prominent lamin phosphorylation sites were generated and validated by sequencing. Growth differences were identified in lamin A/C S22 mutated cells, exhibiting clonal variation independent of the mutation type. The radiosensitivity of these clones was subsequently assessed by colony formation assay, showing that all lamin A/C S22 mutated clones exhibit increased radiosensitivity, though to varying extents. Additionally, S22D mutated cells that mimic persistent phosphorylation at this site present altered lamin A/C localization and aberrant nuclear morphology and structure compared to the parental cells.
Conclusions Studying the interplay between wildtype and mutant lamins and their clonal differences may provide insight into the role of the cancer-specific lamin network in the cellular damage response upon irradiation.
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B-P9

Kinetics of micronuclei formation in NSCLC cell lines after irradiation with photons or ^{12}C ions

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Background Micronuclei (MN) are formed after irradiation (IRR) by induction of DNA damage and resulting chromosome instability. These MN can activate the cGAS-STING signalling pathway, which promotes an anti-tumour immune response by stimulating antigen presenting cells (APCs). The present study investigates the kinetics of MN formation of different NSCLC cell lines after IRR with photons and carbon ions (^{12}C ions), as well as the signalling of APC activation after both types of irradiation. The question is whether ^{12}C ions can induce a stronger immune response than photons.
Materials and methods The NSCLC cell lines A549, H1975 and H1299 were irradiated with photons and ^{12}C ions at doses of 0, 2, 6 or 12 Gy. The MN per cell were then visualised 24, 48 and 72 h after IRR by immunofluorescence microscopy of the dsDNA and the cytoskeleton and quantified using the Imaris software. Components of the cGAS-STING signalling pathway were analysed by qRT-PCR and the secretion of IFN-γ and IFN-β as markers of the immune response were quantified by ELISA. Statistical analysis was performed by ANOVA.
Results Preliminary data show an increase in MN with increasing dose in all cell lines. Especially at the highest dose (12 Gy), ^{12}C ion irradiation led to a more intense induction of MN compared to photons. The number of MN increased up to 3 days after IRR and a decrease of MN was not observed at this time. Initial results of cGAS-STING gene expression show an increase after IRR and a peak on day 2.
Conclusions The results so far suggest that ^{12}C ions may induce a stronger activation of the cGAS-STING signalling pathway due to their higher MN induction, which could enhance anti-tumour immunity. The elicited immunogenic potential allows the improvement of NSCLC therapy by combined immunotherapy and radiotherapy. Further experiments are needed to confirm the differences in the immune response and to identify therapeutic windows and opportunities.
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B-P10

The effect of radiation on cGAS-STING pathway in head and neck squamous cell cancer

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Background Radiotherapy (RT) is an essential part of multidisciplinary therapy for head and neck squamous cell cancer (HNSCC). It has been proven that RT can kill tumor masses directly and also exert pro-inflammatory effects on the body. How to potentiate its immune effect to inhibit tumor development more effectively is drawing increasing attention from researchers and clinicians. RT directly damages the nucleus and chromosomes, causing leakage of DNA components in the cytoplasm, which has the potential to trigger the activation of the cGAS-STING pathway. The latter was identified in 2013, and is an innate immune pathway. This pathway can be activated by abnormal DNA and RNA components

in the cytoplasm, e.g. from viral and bacterial infection, resulting in the expression of multiple interferon-related genes. We now aimed to confirm the activation of this pathway by tumor cell DNA in HNSCC following irradiation and to analyze in detail which immune alterations do result.
Materials and methods For our first in vitro analyses, we used two HPV-negative HNSCC cell lines (HSC4 and Cal33), and the HPV-positive cell line (UMSCC47) as model system. The tumor cells were left untreated, irradiated twice with 5 Gy or a single high dose of 10 Gy, and were then harvested 12 h or 48 h after the last irradiation. Protein extractions were used for Western blot experiments, RNA expression was quantified via real-time PCR, and cell culture supernatants were analyzed for cytokine and chemokine secretion.
Results We found that the expression of cGAS was significantly increased 12 h after irradiation, both at the protein and RNA levels, but then decreases at the 48 h timepoint. In contrast, the expression of STING did not change significantly after irradiation. But, its activated form, Phospho-Sting, was significantly increased 48 h after irradiation of the tumor cells with 2×5 Gy when compared to 10 Gy once or the non-irradiated group, most pronounced in HSC4 cells. The analysis of the supernatant showed that the concentration of IFN-lambda (type III) was significantly upregulated after 2×5 Gy. In contrast, IFN-beta (type I) and IFN-gamma (type II) remained at low levels after irradiation.
Conclusions Hypofractionated irradiation of HNSCC cells activates the c-GAS/STING pathway in HNSCC cells independently of the HPV status and in a dynamic manner. As one immune consequence, we identified for the first-time increased type III interferon secretion. The direct connection to the c-GAS/STING pathway is currently by verified with c-GAS/STING blocking experiments.

B-P11

Radiosensitivity and Radiation Response of Hypoxic Non-Small Cell Lung Cancer Cells

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Background Hypoxia occurs in 80% non-small cell lung carcinoma (NSCLC) cases. Through cellular adaptations and changes of the tumour microenvironment, cellular hypoxia can cause resistance to ionizing radiation. Energetic charged particles with high linear energy transfer (LET) might effectively treat hypoxic solid tumors such as NSCLC. Therefore, we analyzed the radiation response of hypoxic NSCLC cells to high-LET carbon (12C) ions in comparison to X-rays.
Materials and methods A549 (p53 wt) and H358 (p53 null) cells were incubated under normoxia (20% O2) or hypoxia (1% O2) [1] for 48 h and irradiated with X-rays (200 kV) or 12C ions (35 MeV/n, LET ~ 75 keV/µm). Hypoxia was either maintained after irradiation or cells were reoxygenated. Colony-forming ability (CFA) assays were performed 24 h after irradiation (late plating). H2AX immunofluorescence microscopy indicated the cellular response to DNA double-strand breaks (DSBs). Using flow cytometry of DAPI-stained cells, cell cycle progression was evaluated. 4 h post-irradiation, RNA was extracted for RNA sequencing. Cytokine secretion (interleukins IL-6, -8) was measures by ELISA..
Results In both cell lines, hypoxia increased survival after X-rays exposure regardless of continuation of hypoxia after irradiation. 12C ions overcame hypoxia-induced radioresistance [2,3]. After irradiation under hypoxia, cell cycle arrest was less distinct but lasted longer [2,3]. Hypoxia did not significantly influence DSB induction and resolution following irradiation compared to normoxia [2,3]. Hypoxia alone upregulated genes controlling cell migration in A549 cells, and hypoxic response and angiogenesis genes in H358 cells. As expected, a p53 transcriptional response to irradiation was absent in H358 cells but pronounced in A549 cells. Both, radiation and hypoxia, upregulated NF-B target genes, but each a different subset [4]. In A549 cells, the increase in expression of NF-B target genes was greater after exposure to 12C ions in comparison to X-rays [4]. However, under hypoxia, X-rays exposure moreover upregulated genes modulating epithelial-mesenchymal transition (EMT) and immunosurveillance, which was reversed by reoxygenation. Both, radiation and hypoxia, augmented interleukin secretion of both cell lines [4].
Conclusions Regardless of absence of functional p53, hypoxia induced radioresistance. If no fast reoxygenation occurs after irradiation, tumor invasiveness might increase especially after radiotherapy with X-rays. For overcoming hypoxia-induced radioresistance in NSCLC, irradiation with accelerated carbon ions has several advantages based on cell killing and gene expression profile.
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B-P12

Evaluating the FLASH effect in medulloblastoma models with different irradiation modalities

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Background Medulloblastoma is the most common high-grade pediatric brain tumor, treated with surgery, chemotherapy, and craniospinal radiotherapy. Survivors frequently suffer neurocognitive impairments due to healthy tissue damage. Recently, research has focused on FLASH therapy, an experimental technique delivering high-dose irradiation in

very short time (> 40 Gy/s), potentially reducing damage to healthy tissue while maintaining tumor control compared to conventional radiotherapy (convRT). In this study, we irradiated medulloblastoma cell lines and ex-vivo brain slice cultures from healthy mice under FLASH conditions to assess its impact and extent. However, achieving this requires precise adjustment of physical parameters and reproducible dosimetry, which are also key aspects of this study.

Materials and methods This study utilized two different linear accelerators (LINACs): a modified medical LINAC at Skåne University Hospital, Sweden, achieving average dose rates of 400–500 Gy/s, and the ARES LINAC at the Deutsches Elektronen-Synchrotron (DESY), capable of electron energies up to 155 MeV with ultra-short pulses (less than 1 ps in length, compared to 5 ms in convRT). We initially developed and validated dosimetric experiments and irradiation protocols for both LINACs. At Skåne University Hospital and ARES, in-vitro cell cultures, including normal human astrocytes, were irradiated, and cellular survival was assessed using colony formation assays. DNA double-strand break (DSB) repair was analyzed via co-stained γH2AX/53BP1 foci. Additionally, ex-vivo mouse brain tissue was irradiated in Sweden and fixed for DNA repair analysis immediately and 24 h post-irradiation. DSBs in ex-vivo tissue were analyzed using 53BP1 foci. Comparisons were made with convRT.

Results In-vitro studies using LINAC-based FLASH indicated a clear FLASH effect, with similar cellular survival following FLASH irradiation compared to convRT. Ex-vivo experiment analyses are still ongoing to further investigate the potential occurrence of the FLASH effect. At ARES, dosimetric analyses of the electron beam were conducted; however, challenges remain regarding irradiation geometries due to the smaller irradiation fields compared to medical LINACs. Initial in-vitro experiments at ARES emphasized the need for optimized beam setups for homogeneous irradiation.

Conclusions In summary, FLASH irradiation was comparable to convRT in in-vitro studies, while ex-vivo analyses are ongoing to assess the potential protective effect of FLASH radiotherapy. Irradiations at ARES remain challenging; however, with protocol improvements, further in-vitro experiments and eventual ex-vivo studies also including medulloblastoma samples are anticipated in the near future.

B-P13

Dependencies of DNA damage response genes to different types and qualities of ionizing radiation

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Background Radiation quality influences DNA damage complexity and repair. Particle irradiation is used in clinical treatment for its enhanced precision; particularly, high-LET ions such as carbon ions are postulated to overcome resistance by inducing complex, non-repairable DNA damage. Differences in radiosensitivity linked to DNA repair gene loss have recently gained interest [1,2]. One option to exploit such vulnerabilities is synthetic lethality [2,3]. However, a systematic approach for exploring synthetically lethal gene defects across distinct beam qualities is currently missing. This study is the first to employ a CRISPR-Cas9 screen and analysis of DNA repair kinetics to systematically investigate the interplay between radiation type, dose, and DNA repair processes, laying the foundation for precision therapeutic interventions.

Materials and methods The radiation sensitivity of retinal pigment epithelial cells carrying a deletion of tumor suppressor gene TP53 (RPE1 TP53 KO) cells to distinct beam qualities (photon, proton, carbon ions) was determined by a clonogenic survival assay. A custom repair library targeting DNA repair and related processes against 1000 genes pooled was used to identify radiation-specific repair vulnerabilities associated with fractionated radiation. DNA repair kinetics were analyzed for selected knockout cell lines using immunofluorescence microscopy.

Results RPE1-TP53 KO cells exhibited increased radiosensitivity to carbon ions. DrugZ analysis and Wilcoxon Rank-Sum tests revealed radiation type and dose-specific genetic dependencies, with specific dropouts linked to non-homologous end joining and homologous recombination pathways. Hierarchical clustering based on DNA repair gene dependencies identified critical repair factors differentiating radiation modalities, highlighting the complex interplay between radiation type and DNA damage response (DDR) pathways. Selected knockouts analyzed for DNA repair kinetics demonstrated variations in the persistence and resolution of DNA damage foci across radiation types, reflecting differences in repair dynamics and efficiency.

Conclusion This work presents the first pooled CRISPR-Cas9-based screen to identify synthetic lethality in DNA damage response across different radiation qualities. Specific genes conferred DNA repair/survivability associated with certain beam qualities. This offers insights to optimize therapy by combining radiation with DDR/DSB repair-targeting compounds, paving the way to overcome radiation resistance in cancer treatment.

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B-P14

Intensification of proton irradiation of HPV-negative and -positive HNSCC cells by specific targeting of PARP

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Background While for HPV-positive squamous cell carcinoma of the head and neck (HNSCC) a 5-year-survival of 80% is achievable when treated with conventional radiochemotherapy, survival for HPV-negative HNSCC is only 50%. Treatment is accompanied with high normal tissue toxicity. A promising alternative is proton irradiation, due to its improved physical dose distribution and better sparing of normal tissue. The current study examines the effects of proton irradiation on HPV-positive and -negative HNSCC cells, with regard to their variations in radiosensitivity when irradiated with photons. Furthermore, PARP-inhibition will be examined for its radiosensitizing abilities in combination with proton irradiation.

Materials and methods Five HPV-positive and five HPV-negative HNSCC cell lines were treated with photon and proton irradiation (0–6 Gy). Inhibition of PARP was achieved by 1 µM Olaparib or Talazoparib. Treatment

was followed by analysis of clonogenic survival (colony formation assay), DNA damage (53BP1 foci), cell cycle regulation (flow cytometer) and replication stress (DNA fiber assay).
Results HPV-positive cells exhibit a higher radiosensitivity towards proton irradiation than HPV-negative cells and this reflects similarities to photon irradiation. Apart from that, HPV-positive cells do not display increased radiosensitivity towards protons, with a relative biological effectiveness (RBE) of RBE = 1.01, and HPV-negative cells are more sensitive (RBE = 1.14) towards protons. PARP Inhibition increases radiosensitivity, with considerably stronger effects for HPV-positive over HPV-negative cells, and Talazoparib over Olaparib. However, Talazoparib administration is accompanied by high cytotoxicity as demonstrated with an HPV-negative cell line. Cells treated with Talazoparib displayed pronounced replication stress as characterized by dispersed 53BP1 foci in S phase cells as well as enhanced occurrence of replication stops, which are finally leading to a strong G2 arrest.
Conclusions The results suggest a radiosensitivity of HPV-positive cells towards protons similar to that observed after photons. A combination with PARP-inhibition significantly increases the effect of proton irradiation. Due to the cytotoxic effects of Talazoparib especially as observed in HPV-negative tumor cells, further studies should clarify its impact on normal tissue.
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Session C: Cell-extrinsic and systemic factors modulating cellular responses to radiation

a) Key note lecture

C-K1
PRiSM—Personalized Radiotherapy with integrated Scientific Modeling
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Background Radiotherapy is the most applied cancer treatment with over 60% of cancer patients receiving radiation at some point in their clinical care. Yet, we have no biomarker to understand and predict why patients with comparable clinical presentations may have different radiation responses and outcomes. Mathematical and computational models, trained on patient-specific clinical data, can help understand and predict interpatient heterogeneity and personalize radiotherapy protocols.
Materials and methods We deploy different mathematical (ordinary and partial differential equations), spatial statistical (2-point correlation function, power spectral density), computational (agent-based models), machine learning (neural networks, generative adversarial networks), and data-driven approaches to analyze pre-treatment tissue biopsies and radiology images to characterize the spatial architecture of the tumor, immune infiltration, immune interplay, and tumor volume dynamics of different cancers. From these, we can create digital twins and simulate in silico clinical trials to predict response and outcome to different radiation protocols and derive optimal radiation regimens for each patient.
Results Routinely collected tissue biopsies can predict pan-cancer radiotherapy response and outcome with high accuracy [1, 2]. Pre-treatment radiology can predict the optimal radiation fractionation protocol [3], and on-treatment response dynamics can predict the optimal total dose to maximize tumor control and minimize radiation-associated comorbidities [5,6].
Conclusions Integrated scientific modeling is well-positioned to guide clinical decision-making for individual cancer patients [6–10]. Prospective clinical trials will be needed to validate the prediction accuracy of the presented methodologies.

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b) Proffered papers—Oral presentation

C-O1
Influence of HPV status in HNSCC cells on the polarisation of tumour-associated fibroblasts
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Background Head and neck squamous cell carcinomas (HNSCC) develop in the oral cavity, larynx, or pharynx and represent a significant health burden. Increasing evidence suggests that polarisation and radiation-induced senescence of tumour-associated fibroblasts (CAFs) play a critical role in therapy resistance. Studies in rectal cancer indicate that Interleukin-1α (IL-1α)-dependent polarisation into inflammatory CAFs (iCAFs) promotes resistance. This study aimed to evaluate whether similar mechanisms are present in HNSCC and to what extent the HPV status of HNSCC cells affects CAF polarisation.
Materials and methods Cancer-associated fibroblasts (larynx) were stimulated for 24 h with conditioned media from HPV-negative (UPCI-SCC-131) or HPV-positive (VU-SCC-147) HNSCC cell lines. The polarisation of CAFs was assessed using qPCR for markers including CD73, CD90,

Interleukin 6 (IL6), and Leukaemia Inhibitory Factor (LIF). ELISAs were used to measure α-Smooth Muscle Actin and LIF secretion. Stimulation with recombinant IL-1α was used for comparison.
Results Stimulation of CAFs with IL-1α and conditioned media resulted in significant changes in gene expression and LIF secretion. CD73 expression was elevated following stimulation with both IL-1α and conditioned media, with a stronger effect observed for media from HPV-negative cells. IL6, which was not expressed in controls (fresh media), showed dose-dependent upregulation after IL-1α stimulation and slight expression following stimulation with conditioned media. LIF exhibited a dose-dependent increases at both the gene and protein levels in response to IL-1α and conditioned media stimulation, with significantly higher levels observed for HPV-negative cells compared to HPV-positive cells ($P = 0.01$). α-SMA was exclusively detected after stimulation with conditioned media.
Conclusions The results suggest that conditioned media from HNSCC cell lines can induce both inflammatory ($IL6^{high}$, LIF^{high}) and myofibroblastic ($IL6^{low}$, $\alpha\text{-SMA}^{high}$) CAF phenotypes. In comparison to media from HPV-positive cells media from HPV-negative cell lines appeared to have a stronger regulatory effect in marker expression.
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C-O2

Modulation of T cell activation in HNSCC through combined DNA damage response inhibition, radiotherapy, and PD-1/PD-L1 axis inhibition

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Radiation Oncology 2025, **20(s1)**:C-O2

Background The treatment of head and neck cancer poses a therapeutic challenge. Human papilloma virus (HPV)-negative tumors show increased radioresistance, which has a negative impact on the efficacy of radiotherapy (RT) and thus on the prognosis. Clinical trials are investigating whether the combination of DNA damage repair inhibitors (DDRi) and RT can help overcome radioresistance. However, little is known about the potential of this combination to modulate the immunogenicity of tumor cells. Preclinical data, combining RT with either AZD0156 (ATM inhibitor) or VE-822 (ATR inhibitor) show that the immunophenotype of HNSCC cells is altered [1]. Both, ATM and ATR, are central players in DNA double-strand break repair via homologous recombination or non-homologous end-joining. We now investigated in a co-cultivation setup, whether the treatment-modified immunophenotype of HNSCC cells affects the activity of human CD8-positive T cells.
Materials and methods HPV-positive and HPV-negative HNSCC cells were seeded and treated after 24 h with DDRi (AZD0156 or VE-822). This was followed by hypofractionated RT with 1 × 5 Gy on the same day and after 24 h. In parallel to the first RT, human CD8 + T cells were isolated from a healthy donor using MACS beads, CFSE-stained and pre-stimulated for 48 h in a CD3/CD28-coated plate. 24 h after the second RT, the T cells were harvested and added to the treated tumor cells in a 1:1 ratio. The cells were co-cultured for 96 h and finally harvested. The expression of CD3, CD8, CD25 and HLA-DR on the T cells was determined by flow cytometry.
Results The co-cultivation of CD8 + T cells with irradiated HPV-negative cells led to an increase in the proliferation of the T cells. Pretreatment with RT + ATRi led to a significant increase in proliferation of HSC4 compared to RT or RT + ATMi. This combination also led to a significant increase in the expression of HLA-DR on the examined T cells after co-cultivation. However, treatment of the T cells in co-culture with pembrolizumab, nivolumab or durvalumab had no effect.
Conclusions Treatment of HNSCC tumor cells with RT + DDRi alters their immunophenotype. CD8 + T cells respond with increased proliferation, especially in co-culture with treated HPV-negative cells. The HLA-DR and CD25 status is also influenced by this. The secretion of INFγ is currently being investigated.
Acknowledgements This work was partly funded by the IZKF Erlangen.
Trial registration and/or ethics approval number All results are covered by the ethical approval of the IMMO-NHD trial, and written informed consent was obtained from all donors. Approval was granted by the institutional review board of Friedrich-Alexander-Universität Erlangen-Nürnberg on November 9, 2022 (application number 21-415-B).

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C-O3

Effect of ^{12}C-ion irradiation in combination with PD-L1 inhibitors in lung tumor cells and tissue

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Radiation Oncology 2025, **20(s1)**:C-O3

Background Carbon (^{12}C) -ion irradiation leads to more efficient killing of tumor cells and better protection of normal tissue through precise dose application and increased biological efficacy. Previous work has shown that irradiation can upregulate the immune checkpoint molecule PD-L1 in NSCLC and thus influence T-cell-mediated immune responses. The current aim is to determine whether PD-L1 and its binding immune cells influence NSCLC survival after ^{12}C-ion or photon irradiation.
Materials and methods A549 and PD-L1 knockout ($A549^{PD-L1KO}$) cells were irradiated with 2, 6 or 12 Gy ^{12}C ions or photons. In vitro monocultures of the cells and cocultures with Jurkat cells were performed under IFNγ stimulation, α-PD-L1 antibody addition or the combination. PD-L1 expression was examined by flow cytometry and tumor cell survival in the colony formation assay.
Results ^{12}C ions and photons increase PD-L1 receptor expression in A549 in a dose- and time-dependent manner, which is further enhanced by IFNγ. Cellular survival of untreated A549 and $A549^{PD-L1KO}$ in monoculture does not differ after irradiation. Treatment with IFNγ has a radiosensitising effect on A549. This effect is attenuated by the addition of α-PD-L1. These effects are not observed in $A549^{PD-L1KO}$. Compared to monoculture, coculture only has a radiosensitising effect on $A549^{PD-L1KO}$.
Conclusions The results indicate that PD-L1 surface expression in the presence of immune cells has an influence on the cellular survival of A549. For further validation, the radiation-induced DNA damage will be visualised in patient samples in an ex vivo assay using the γH2AX/53BP1 foci technique.
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c) Proffered papers—Poster presentation

C-P1

Characterization of circulating tumor cells in an osteosarcoma mouse model: isolation, gene expression, and functional properties

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Radiation Oncology 2025, **20(s1)**:C-P1

Background Circulating tumor cells (CTCs) are pivotal in the process of metastasis and hold significant potential as biomarkers for monitoring tumor progression and evaluating response to radiation therapy. However, their isolation and characterization remain challenging. In this study, we isolated and cultured CTCs from mice inoculated with an osteosarcoma cell line. We aimed to validate their tumor origin, assess their gene expression profiles, and identify key molecular changes that enable primary tumor cells to enter the bloodstream and form metastases.
Materials and methods CTCs were isolated from the blood of osteosarcoma-bearing mice and cultured. To confirm their origin, we analysed the expression of osteosarcoma specific markers and performed immunohistochemistry. RNA sequencing and differential gene expression analysis were conducted to compare the parental cell line with the CTC-derived cell lines, followed by Gene Ontology (GO) and KEGG pathway enrichment analyses. Additionally, in vitro migration assays were performed to evaluate CTC motility. Furthermore, clonogenic survival assays after irradiation were conducted for all CTC cell lines and the parental cell line.
Results The CTC cell lines maintained an osteosarcoma-specific gene expression profile, confirming their tumor origin. Gene expression analysis identified 1249 differentially expressed genes between parental and CTC cell lines. GO analysis revealed significant enrichment in extracellular matrix remodeling, antigen processing, and metabolic pathways. Col6a1, an extracellular matrix component associated with osteosarcoma metastasis, was significantly upregulated in CTCs. KEGG analysis highlighted upregulation of genes involved in MHC class I and II antigen presentation, suggesting potential immune evasion mechanisms. CTCs also expressed von Willebrand factor (Vwf), which may facilitate platelet-mediated immune protection and endothelial adhesion. Functional assays demonstrated that CTCs exhibited a significantly enhanced migratory capacity and a reduced nuclear size compared to the parental cell line, further supporting their metastatic potential. No difference in survival after irradiation was observed between parental and CTC cell lines.
Conclusions Our study provides a comprehensive characterization of osteosarcoma-derived CTCs, highlighting their transcriptional and functional heterogeneity. We identified potential molecular pathways involved in their generation and survival in circulation, including extracellular matrix remodeling, antigen processing, and immune evasion. Notably, Col6a1 and Vwf may play critical roles in osteosarcoma metastasis formation. The elevated MHC I expression in CTCs is an interesting observation, as radiotherapy is known to enhance MHC I expression in primary tumors.
Trial registration and/or ethics approval number "Regierungspräsidium Darmstadt Dezernat V 54–Veterinärwesen und Verbraucherschutz" (protocol code Da17/2000, 22 January 2021 and Da17/2005, 19 September 2023).

C-P2

Investigations on the activation of the complement system as part of the radiation response of the tumor microenvironment

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Background The complement system plays a crucial role in the tumor microenvironment, exhibiting both anti-tumor and pro-tumorigenic effects across different tumor types. A tumor-promoting role has been demonstrated in a mouse sarcoma model [1], whereas in melanoma, X-ray irradiation has been shown to activate the complement system and contribute to tumor inhibition [2]. We aimed to investigate the impact of irradiation on the immune-modulating effects of the complement system in the sarcoma model above. Since hypoxia is a hallmark of sarcomas and can influence complement regulation in the tumor microenvironment [3], we first explored the interplay between irradiation, complement activation, and oxygen levels in sarcoma cells in vitro. Particular focus was placed on the expression of key hypoxia-inducible factors such as CXCR4, a chemokine receptor involved in tumor progression [4]. Given the growing clinical interest in carbon ion therapy for sarcoma, we compared the effects of X-ray and carbon ions.
Materials and methods MN/MCA1 murine sarcoma cells were exposed to X-rays or carbon ions (75 keV/µm—MIT) after cultivation under normoxic (21% O_2) or chronic hypoxic (1% O_2) conditions for two weeks. All cells were harvested and irradiated under normoxic conditions and subsequently kept in normoxic or hypoxic culture for 1–2 days with same oxygen levels as before irradiation. We assessed cell survival and migration (Boyden chamber) and analyzed the expression of complement factors (C3, C3a), immunogenic cell death markers (DAMPs), and CXCR4 using flow cytometry and ELISA.
Results Our findings show that cell migration and CXCR4 expression were more pronounced following X-ray irradiation compared to carbon ion exposure, with a stronger effect on migration under hypoxic conditions. Notably, CXCR4 expression remained low and was not significantly influenced by hypoxia alone. The observed lower induction of CXCR4 after carbon ion irradiation aligns with hypoxia-induced radioresistance generally being acknowledged as less pronounced with carbon ions than with X-rays [5]. Additionally, we observed a radiation-induced increase in DAMP release and complement activation, without significant differences between X-rays and carbon ions.
Conclusions To determine the biological consequences of radiation-induced immune activation (complement activation, DAMP release) and CXCR4 expression in vitro, we are investigating tumor growth, vascularization, and metastasis in the corresponding sarcoma mouse model (MN/MCA1 tumors) following X-ray and carbon ion irradiation at SIS/GSI.
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All animal experienced were performed in accordance with German federal law and approved by the local authorities under the registration number DA17/2007.

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C-P3

RIG-I pathway as a key regulator for radiotherapy-induced anti-HNSCC T cell responses

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Background Alcohol and tobacco abuse as well as the infection with the human papilloma virus are the two main risk factors for the development of head and neck tumors (HNSCC). These tumors are therefore differentiated into HPV-positive and HPV-negative ones and interestingly differ in their radiation-sensitivity. Radiotherapy (RT) causes DNA damage in irradiated cells, which has effects on the immune system that can be suppressive or stimulating. Immune stimulations as the release of nucleic acids from the nucleus into the cytoplasm cause the activation of pattern recognition receptors (PRR) as part of the innate immune system. The PRR "Retinoic acid-inducible gene 1" (RIG-I) is activated upon its recognition of double-stranded RNA in the cytoplasm. Therefore, activation of RIG-I after RT can potentially modulate the tumor cell immunophenotype and the T cell immune response via the release of type 1 interferons such as Interferon-β. Since conflicting effects of Interferon-β on the anti-tumor T cell response have been described, it is important to determine to what extent RIG-I activation in irradiated HNSCC cells influences the adaptive anti-tumor immune response.
Materials and methods Two HPV-positive and two HPV-negative HNSCC cell lines were used. Cell lines were treated with either hypo-fractionated RT (5×3 Gy) or one single high dose (19.3 Gy). Abundance of RIG-I and associated proteins in the cytoplasm of the irradiated cells was determined by Western blot. In the HPV-negative cell line HSC4, RIG-I was also investigated several days post-RT by intracellular staining and subsequent flow cytometry.
Results RT, especially hypo-fractionated irradiation, increases the amount of RIG-I in three out of four HNSCC cell lines. Specifically, in HSC4 a significant increase of RIG-I abundance can be seen. This was also confirmed using flow cytometry. The RIG-I activating E3 ligase RNF135 is modulated opposite to RIG-I after irradiation. Additionally, RIG-I remains elevated or even increases until multiple days after irradiation.
Conclusions Our results indicate that RIG-I plays a key role in irradiation-induced changes of the HNSCC immunophenotype and strengthen the assumption of a possible T cell immunomodulation post RT.
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C-P4 Relevance of biological differences in the DNA damage response to proton vs. photon irradiation for radiation quality-guided combination strategies

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Background Advancements in clinical radiation therapy (RT) focus on enhancing tumor control while minimizing collateral damage to healthy tissues. Proton beam therapy, renowned for its unique physical properties, has emerged as a promising treatment modality. Emerging evidence, including studies from our group, highlights biological differences in the relative importance of specific DNA double-strand break (DSB) repair mechanisms elicited in response to proton beam irradiation compared to X-ray photon irradiation. This study aimed to gain deeper insight into the interplay between radiation quality, DNA repair deficiencies, and radiosensitivity in cancer cells exposed to Spread-Out Bragg Peak (SOBP) protons versus X-ray photons.
Materials and methods Syngeneic cell lines with or without DNA repair protein deficiencies (A549-WT, A549ATM-knockout, HCT116WT, and HCT116BRCA2-knockout) were utilized to investigate the role of homologous recombination (HR) and radiosensitivity. The U2OS reporter system DRGFP was employed to assess HR pathway activity. In addition, chromosomal repair kinetics was determined by using classical Cytogenetics in both HR-proficient and HR-deficient cell lines. Finally, the radiosensitizing effects of combining an ATM inhibitor with SOBP proton versus X-ray irradiation were evaluated in DNA repair-proficient cancer cell models.
Results The reporter system results indicated a higher activation level of HR in response to SOBP proton radiation compared to photon radiation. In addition, HR-deficient cancer cells exhibited heightened radiosensitivity and delayed chromosomal repair kinetics following SOBP proton irradiation compared to X-ray irradiation pointing to a use of HR defects as biomarkers for patient stratification. Proof-of-concept colony formation experiments combining ATM inhibitors with SOBP proton irradiation demonstrated significantly enhanced radiosensitivity in DNA repair-proficient cells compared to combined inhibitor-photon irradiation as demonstrated by significantly reduced survival fractions. These findings point to a high therapeutic potential of combined treatment with SOBP proton irradiation and inhibitors of specific DNA repair pathways.
Conclusions Our results demonstrate elevated reliance of cancer cells of HR for repairing DSBs induced by SOBP proton irradiation compared to X-ray photon irradiation, thereby corroborating relevant biological differences in DNA repair mechanisms. Future studies are needed to validate the use of DNA repair inhibitors for enhancing the treatment efficacy of SOBP proton irradiation in vivo and thereby paving the way for a future biology-driven proton radiotherapy individualization.
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C-P5 Unravelling B cell dynamics in a multimodal radioimmunotherapy approach with a whole tumour cell vaccine

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Radiation Oncology 2025, **20(s1)**:C-P5

Background Recent studies highlight promising outcomes in combining radiotherapy (RT) with immunotherapies for cancer treatment. Our research focuses on developing whole-tumour cell vaccines created using high hydrostatic pressure (HHP) to inactivate tumour cells. Previously, these vaccines showed improved local and abscopal tumour control when combined with hypofractionated RT and α-PD1 antibodies, associated with a robust T-cell response. B cells have a complex role in cancer, with both pro- and anti-tumorigenic effects observed. Their role in the anti-tumour immune response in such a multimodal radioimmunotherapy approach remains unknown.
Materials and methods In a bilateral tumour model, B16 melanoma cells were injected into C57BL/6 mice, with only the primary tumour receiving local irradiation (2×8 Gy). Immune checkpoint inhibitors (ICI) were administered, including two doses of α-CTLA-4 followed by α-PD1. HHP

vaccines were locally injected at the primary tumour site, with or without adjuvants (poly I:C and α-CD40). Tumour growth and survival were monitored. Spleens and lymph nodes were analysed for B-cell immune responses by multicolour flow cytometry, and serum antibody titres were measured using ELISA.
Results The combination of RT, ICI, and HHP vaccine with adjuvants demonstrated the best tumour control and survival. This approach caused a dynamic shift in B cell populations, including follicular B cells and antibody-secreting cells, with an initial decrease on day 4 post-RT, followed by a significant increase in draining lymph nodes on day 7. Notably, adjuvants enhanced IgG concentration in the serum on day 14.
Conclusions These results suggest that combining RT, ICI, and HHP vaccines with adjuvants offers a promising synergistic approach to cancer treatment. The findings provide initial insights into the dynamics of B cell populations and antibody secretion in a multimodal radioimmunotherapy approach. However, the complex roles of B cells in cancer warrant further research to clarify the mechanisms behind these effects and whether the secreted antibodies are tumour-specific.
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Trial registration and/or ethics approval number RUF-55.2.2-2532-2-1950-19.

C-P6

Spatio-temporal dynamics of radiation-induced senescence and their relevance for normal tissue toxicity

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Radiation Oncology 2025, **20(s1)**:C-P6

Background For effective and tolerable tumor therapies, the simultaneous consideration of the radiation response in tumor and normal tissue is essential. Herein radiotherapy (RT)-induced senescence of lung epithelial cells and the development of a senescence-associated secretory phenotype determine normal tissue toxicity after thoracic irradiation. As part of the BMBF-funded SeniRad consortium, this study introduces first investigations concerning the spatio-temporal dynamics and the underlying signaling networks of radiation-induced senescence in normal tissue cells in vitro, ex vivo and in vivo.
Material and methods The mechanisms of senescence induction is analyzed in a time- and dose-dependent manner in normal epithelial cells, fibroblasts, and endothelial cells. This includes classical cell culture assays for senescence, proliferation, viability, cell death, DNA damage, and DDR, along with expression analyses of SASP factors at the RNA (RNAseq, and qRT-PCR) and protein (Western Blot) levels. Parallel in vivo investigations include the time-resolved and cell-type dependent senescence induction following whole thorax irradiations (WTI mouse model). Therefore, currently methods are being validated, optimized and harmonized among the members of the consortium.
Results Our first results indicate that normal epithelial cells undergo senescence via the p53/p21 signaling pathway (96 h time point), while fibroblasts do not seem to use this signaling pathway. The time-resolved, analysis of the different normal cell types is ongoing and respective results will be presented. Likewise, the results concerning the currently performed analyses of already obtained lung tissue samples from our murine in vivo model of radiation-induced pneumopathy will be presented.
Conclusion A time-resolved characterization of the phenotypes of radiation-induced senescence in normal epithelial cells as well as neighboring endothelial cells and fibroblasts and the identification and functional validation of relevant regulatory signaling cascades and pathology-associated effector molecules aims to provide a molecular basis for developing pharmacological strategies that enable targeted protection against radiation-induced damage in normal tissues by modulating senescence whilst maintaining anti-tumor activity of radiotherapy.
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C-P7

Radiotherapy modulates the migration of neutrophilic granulocytes in a preclinical approach for breast cancer

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Radiation Oncology 2025, **20(s1)**:C-P7

Background The neutrophil–lymphocyte ratio is an important prognostic marker associated with overall survival in mammary carcinoma (MC). Neutrophils play a controversial role in the tumor microenvironment and can exhibit anti- and pro-tumorigenic properties. The biological mechanisms by which neutrophils influence MC and migrate into the tumor site are still poorly understood. In this project, we investigate whether chemokines or small molecules influence the migration behavior of neutrophils and whether irradiation has an effect. The aim is to better understand neutrophils and their functionality as prognostic markers.
Materials and methods The secretomes of different MC cell lines were consolidated in irradiated (8 Gy) or non-irradiated condition to stimulate neutrophil migration. The chemokines in the secretomes were determined by multiplex cytokine measurement and separated by 3 kDa filtration into the protein fraction and the nucleic acid/small molecule fraction. The functionality and activity of neutrophils was analyzed by a transwell migration assay and flow cytometry.
Results No differences in the chemokine concentration of IL-8, ENA-78 and other chemokines were observed between irradiated and non-irradiated conditions within the same cell lines. However, the chemokine concentration varied between the cell lines. It was also observed in the cell lines that the migration of neutrophils is stimulated by the protein fraction of the secretome. The migration of neutrophils towards the non-irradiated protein fraction is significantly higher compared to the irradiated protein fraction in the secretome of MDA-MB-231 cells. In addition, it was observed that the degranulation of neutrophils increases by the secretome of irradiated MC cells.
Conclusions The migration behavior of neutrophilic granulocytes is modulated by secreted proteins from MC cells. In addition, the activity of neutrophils was observed to increase upon irradiation of MC cells. The modulation of neutrophils by irradiated tumors is being investigated further in order to evaluate neutrophils more precisely as a prognostic marker in the future.

C-P8

Comparison of two triple therapy regimens involving RT and anti-PD-1 in tumor-bearing mice

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Background Local radiotherapy (RT) has the potential to elicit T cell-dependent regression of distant, non-irradiated tumor sites—a phenomenon known as the abscopal effect—particularly when administered alongside immune checkpoint inhibitors (ICIs). Nonetheless, such responses remain infrequent in clinical settings, underscoring the need

for more effective strategies. In this study, we evaluated and compared two combinatorial approaches—RT plus αPD-1 and αCTLA-4 versus RT plus αPD-1 and IL-2/αIL-2 immune complexes (IL-2c)—with the aim of enhancing systemic anti-tumor responses.
Materials and methods In murine models bearing bilateral subcutaneous C51 colon carcinoma or B16 melanoma, localized irradiation of the primary tumor was administered in two fractions of either 8 Gy or 12 Gy. Treatment with αPD-1 and αCTLA-4 antibodies was initiated concurrently with RT and continued on a weekly schedule, while IL-2/αIL-2 complexes (IL2c) were administered intraperitoneally for three consecutive days, beginning on day 3 following RT. Tumor progression and overall survival were monitored, and the frequency of tumor-specific $CD8^+$ T cells was analyzed via flow cytometry using MHC class I tetramers in combination with lineage and activation markers. Furthermore, the functional profiles of $CD8^+$ and $CD4^+$ tumor-infiltrating lymphocytes (TILs), along with activated dendritic cells (DCs), were systematically evaluated.
Results The abscopal response was markedly enhanced in mice receiving the αCTLA-4-based triple combination therapy (n = 13) compared to those treated with the IL2c-based regimen (n = 9) ($p < 0.05$), as well as in comparison to hRT combined with either αPD-1 alone (n = 8) or αCTLA-4 alone (n = 10) ($p < 0.05$). This αCTLA-4-containing triple combination significantly prolonged survival in both tumor models and led to complete tumor rejection in 8 out of 13 mice within the C51 model. T cell depletion with hRT + αPD-1 + αCTLA-4 group demonstrated that control of the irradiated tumor primarily relied on $CD8^+$ T cells. In contrast, the regression of non-irradiated tumors was only partially dependent on $CD8^+$ T cells and required a significant contribution from $CD4^+$ T cells as well. Functional analysis of T cells indicated that hRT + αPD-1 + αCTLA-4 (n = 9) elicited higher frequencies and absolute numbers of polyfunctional tumor-specific $CD8^+$ T cells ($TNF\alpha^+$ $IFN\gamma^+$), $CD4^+$ effector T cells ($TNF\alpha^+$ $IFN\gamma^+$ and $IFN\gamma^+$ $IL\text{-}2^+$), and more CD80 + CD86 + expression on CD103 + DCs compared to hRT + αPD-1 + IL-2c treatment (n = 9) ($p < 0.05$), with these effects being especially pronounced in non-irradiated tumors..
Conclusions hRT + αPD-1 + αCTLA-4 elicited a greater accumulation of cytotoxic effector TILs compared to the hRT + αPD-1 + IL-2c treatment, thereby amplifying the abscopal response and resulting in a substantially higher rate of complete tumor regression at distant, non-irradiated sites.

C-P9

Sex- and age-specific differences in the immune response of macrophages after low-dose exposure to X-rays

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Background Radiation protection measures are of utmost importance to minimize health risks among exposed individuals. Beside causing DNA-damage, ionizing radiation can also trigger the immune system. Although differences of the general immune response between sexes and age-groups are known, they have been insufficiently investigated for consideration in radiation protection measures until now. Thus, current regulations originate almost exclusively from data derived from healthy, middle-aged males. Since macrophages (MPHs), as key mediators of inflammation, can strongly influence the immune response after low dose radiotherapy (LD-RT), they display an interesting target for further research. Consequently, gender-specific differences of MPH-plasticity and -functionality after LD-RT in vitro were investigated.
Materials and methods Wild-type C57BL/6 (WT) mice of different sex and age (6 weeks and 14 weeks) were used to isolate monocytes, which were differentiated into the MPH subtypes by the addition of cytokines (M0 + MCSF; M1 + GMCSF, LPS, IFNγ; M2 + MCSF, IL4). Irradiation was carried out using X-rays (0; 0.1; 0.5; 1 and 2 Gy). The phenotype was examined by analyzing surface markers CD80, CD86, CD206, MHC-II and PD-L1 via flow cytometry. In addition, arginase activity, the amount of reactive oxygen species (ROS) and the secretion of pro- and anti-inflammatory proteins were examined.
Results ROS analysis revealed a decrease of ROS levels with increasing irradiation doses, whereby the levels of male-derived MPHs peaked at 0 Gy and from females at 0.1 Gy. A significant reduction of pro-inflammatory proteins such as iNOS, IL23 and IL15 after irradiation with 0.5 Gy could only be detected in female M1 MPHs. The surface marker analysis showed only little changes. Noteworthy, there was a significant decrease of CD206 (anti-inflammatory) in female M1 MPHs and of CD86 (inflammatory) in male M1 MPHs after irradiation with 1 or 2 Gy. Further, first results of the second age group (14 weeks) showed significant differences between the age groups in the basal expression of MHC-II and CD86.
Conclusions The immune alterations of MPHs after LD-RT is influenced by gender. Further investigations will elucidate how the age influences the response and which mechanisms are involved. This work will contribute to the expansion of current protection measures and treatment regimens and thus potentially provide a support for the individualization of present regulations.
Acknowledgements Supported by the German BMFTR (TOGETHER, 02NUK073).
Trial registration and/or ethics approval number C57Bl/6 mice were ordered from Janvier Labs (Le Genest-Saint-Isle, France) and maintained in a SPF facility under sterile atmosphere at the animal facility of the Universitätsklinikum Erlangen (Franz-Penzoldt-Center). The animal procedures have been approved by the "Regierung of Unterfranken" (Approval Numbers: TS-9/2022 from January 1st 2022 and TS-4/2025 from May 1st 2025) and they were conducted in accordance with the guidelines of Federation of European Laboratory Animal Science Associations (FELASA).

C-P10

Analyses of the effects of radiotherapy and cannabidiol (CBD) on the immune phenotype of breast cancer cells

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Background Due to the lack of estrogen, progesterone, and HER2 receptors, for triple-negative breast cancer (TNBC) limited treatment options other than chemotherapy and radiotherapy (RT) remain. However, its potential immunogenicity makes immune therpay an important alternative in the management of this malignancy, particularly for patients with high expression of immune checkpoint molecules (ICMs), such as PD-L1 and with higher number of tumor-infiltrating lymphocytes (TILs). The poor prognosis of patients with increased radioresistance and weak response after immunotherapy with immune checkpoint inhibitors calls for new combined treatment, including the non-psychotropic cannabidiol (CBD). In this regard, reactive oxygen species (ROS) might play a role causing harm including DNA and cellular damage or acting as crucial signaling molecules in the immune system. So far, little is known about the immunological impact on breast cancer cells after RT in combination with CBD.
Materials and methods MDA-MB-231(human TNBC cell line), MCF-7(ER+, PR+) and 4T1(murine TNBC cell line) were treated by a gradient concentration of CBD (0 µM, 3 µM, 5 µM, 7 µM, 10 µM). Cytotoxic effect was detected at 24 h, 48 h and 72 h after CBD administration. Tumor cell death forms and the expression of immune checkpoint molecules (ICMs) on the three breast cancer cell lines after the combination of RT (2 × 5 Gy or 10 Gy) with CBD 3 µM were tested by using multicolor flow cytometry. The cytoplasmic ROS was measured via 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) staining on Tecan—Spectrophotometer plate reader.
Results CBD induced significant cytotoxicity in breast cancer cells in a time- and concentration-dependent manner. A significant higher cytotoxicity was observed in the combined treatment—CBD (3 µM) plus RT (2 × 5 Gy or 1 × 10 Gy) in all three examined breast cancer cell

lines compared to the treatments of RT or CBD only. Particularly significant higher levels of secondary and primary necrotic tumor cells were observed in TNBC cells. Regarding the expression of ICMs, a highly dynamic and individual modulation can be observed. Further, CBD plus RT induced more ROS in comparison to CBD (3 µM) alone or RT (2 × 5 Gy or 1 × 10 Gy).

Conclusions The combination of CBD with RT has a significant cytotoxicity on breast cancer cells, particularly on the tested TNBC cell lines (MDA-MB-231 and 4T1). Further, CBD alone and in combination with RT does exert immune modulation by individually impaction on the expression of ICMs. Mechanistically, ROS might be one key regulator of immunological effects of CBD/CBD + RT on breast cancer cells.

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C-P11

Investigation of gender-, age-, and inflammation-dependent differences in murine skin and lung fibroblasts after radiation exposure

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Background In any case of radiation exposure, be it radiotherapy or nuclear accident, the skin is exposed to a significant dose of radiation by different radiation qualities which can impair its regenerative capacity and alter the cellular environment. In particular, fibroblasts respond to this radiation exposure by modifying their function. Radiation-induced damage can lead to an overproduction of collagen, which is referred to as fibrosis and negatively impacts wound healing. These changes in skin structure can lead to chronic issues that may manifest differently depending on age, gender, and inflammatory condition. The aim of this study is to investigate the gender-specific effects of low- and high-dose single exposures on healthy as well as inflammatory fibroblasts in the skin, ears, and lungs of various age groups in order to further individualize radiation protection measures by identifying risk groups and markers.

Materials and methods Isolation of murine skin and lung fibroblasts from male and female mice (C57BL/6) of various age groups; induction of inflammation via TNF-alpha. Irradiation using 120 kV X-rays (0; 0.1; 0.3; 0.5; 1.0 and 2.0 Gy) followed by analysis of fibroblast-specific markers and determination of cell death using flow cytometry after 24 and 72 h. Investigation of radiation-induced fibrosis using SirCol™ and hydroxyproline assays, as well as qPCR and ELISA analyses.

Results Preliminary results show a difference in the expression of CD87, CD44 and Fibroblast Activation Protein FAP between healthy and inflammatory tissue. Additionally, tissue-specific variations and an increased number of fibroblasts in inflammatory conditions can be observed.

Conclusions The aim of this study is to fill gaps in the current knowledge regarding gender- and age-specific biological effects after ionizing radiation. For this reason, it is important to clarify potential effects in the mentioned conditions in normal tissue, and especially in the skin, as it is the organ most commonly affected by any type of radiation exposure.

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Trial registration and/or ethics approval number C57Bl/6 mice were ordered from Janvier Labs (Le Genest-Saint-Isle, France) and maintained in a SPF facility under sterile atmosphere at the animal facility of the Universitätsklinikum Erlangen (Franz-Penzoldt-Center). The animal procedures have been approved by the "Regierung of Unterfranken" (Approval Numbers: TS-9/2022 from January 1st 2022 and TS-4/2025 from May 1st 2025) and they were conducted in accordance with the guidelines of Federation of European Laboratory Animal Science Associations (FELASA).

C-P12

The gut microbiome as a biomarker for space radiation risk

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Radiation Oncology 2025, **20(s1)**:C-P12

Background Radiation remains one of the most significant obstacles for space missions. With upcoming deep space missions to the Moon and Mars, radiation exposure will increase dramatically compared to missions in low-Earth orbit, such as those to the International Space Station. This project aims to investigate the effect of radiation on the composition and diversity of the gut microbiome in mice. Our goal is to identify key microbial signatures that could help predict radiation-related health risks and inform the development of personalised countermeasures to better protect astronauts during long-duration interplanetary missions.

Materials and methods In this pilot cohort, a genetically modified mouse model with a predisposition for colorectal cancer (C56BL/6 J-Apc$^{Min/+}$, n = 8) and wild type mice (C56BL/6 J, n = 8) were exposed to 5 Gy X-rays (250 kVp) whole body irradiation, including sham irradiated control groups. Faecal pellets were collected throughout the course of the experiment and 16S rRNA sequencing was performed to investigate changes in the microbiome composition and diversity between the different treatment groups. All mice were sacrificed 30 days post irradiation and tumours were quantified in the different sections of the gastrointestinal tract (GIT), spanning from the duodenum to the colon, excluding the cecum, followed by histological H&E stainings to determine the crypt and villi parameters.

Results Preliminary results indicate that, 30-days post irradiation, tumour counts in the GIT of C56BL/6 J-Apc$^{Min/+}$ mice were 56 ± 20 for 0 Gy and 35 ± 20 for 5 Gy (average ± standard deviation), indicating a decrease after irradiation. The frequency and size of the GIT tumours will be analysed to understand whether or not radiation had an impact on the growth rate of the tumours. In addition, these results will be linked to the results of the 16S rRNA sequencing (ongoing) to investigate how the composition and diversity of the microorganisms in the gut changed after radiation exposure and during tumour development in both the C56BL/6J -Apc$^{Min/+}$ and C56BL/6J mice.

Conclusions The higher tumour burden in the non-irradiated C56BL/6 J-Apc$^{Min/+}$ mice compared to the irradiated group was surprising, since previous research showed a statistically significant increase in the number of GIT tumours post irradiation of 5 Gy. In addition to the described 30 days follow-up in this cohort, a 90-day follow-up cohort will be done later this year to understand if radiation causes a latency in tumour development in the C56BL/6J-Apc^{Min+} mice.

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Trial registration and/or ethics approval number This project entitled MICROBIAN (The gut MICRObiome as a Biomarker for spAce radiatioN risk) is accepted according to the German federal law and approved under the Hessen Animal Ethics Committee (Project license DA17/2010).

C-P13

Impact of age and biological sex on the radiation mediated response in osteoclasts after low-dose exposure with X-ray in a murine model

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Background Low-dose radiation therapy is utilized as an alternative therapy of degenerative joint diseases of the bone, where manifold cell types of the immune system and cells of the bone are involved, e. g. the osteoclasts (OC) with bone-resorbing characteristics. However, the mechanistic details of the radiation-mediated effects in this therapy are not uncovered yet nor the impact on the healthy organism in terms of radiation protection. Indeed, exposure to low-dose X-ray radiation happens recurringly on multiple occasions (medicine and research, sources present in nature, human-built products), but fundamental data is lacking to claim accurate thresholds and recommendations for individuals. Thus, the effects on OC after exposure with X-ray in the low- to high-dose range were investigated with regard to age, sex and inflammation background.
Materials and methods Bone marrow from hind legs of six and 14 weeks old (w) C57Bl/6NRj mice (both sexes) was taken to generate OC via cytokine stimulation (RANK-L 50 ng/mL, M-CSF and optionally TNF-α 10 ng/mL). Cells were exposed to X-ray radiation with single doses of 0, 0.1, 0.3, 0.5, 1, 2 Gy or the fractionated doses (2×0.05, 2×0.15, 2×0.25, 2×0.5, 2×1 Gy). Reactive oxidative species (ROS) were assessed from OC via flow cytometry as a measurement of total cell stress. Moreover, supernatants and RNA were taken to monitor secreted cytokines, proteins and gene-specific activation via ELISA and qPCR. Besides, we used TRAP staining to observe the differentiation patterns of OC and their precursors.
Results A sex-opposing trend emerges in the differentiation pattern of OC, because the number of OC decreases in the female group with increasing dose, while it increases in the male one. In the younger female cohort, ROS levels significantly decrease with increasing dose accompanied by significant differences between the inflammatory and healthy cohorts. In contrast, ROS levels and OC activation increase with higher doses in the older female age group. Whereas the activation pattern of the young cohorts shows only slight deviations after exposure. Previous results indicate no significant impact by the radiation fractionation. Final experiments are conducted.
Conclusions While differences between the doses in ROS levels were mainly observed at different age cohorts (14 w increase at 1 and 2 Gy; 6 w decrease at 0.5, 1 and 2 Gy), the differentiation of OC shows mainly sex-specific effects where a significant increase in OC number at 2 Gy was observed in the male cohort. These results indicate an influence of age, sex and inflammatory status in the radiation response of murine OCs and thus, insights into systemic factors of the cellular response to radiation.
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Trial registration and ethics approval number C57Bl/6 mice were ordered from Janvier Labs (Le Genest-Saint-Isle, France) and maintained in a SPF facility under sterile atmosphere at the animal facility of the Universitätsklinikum Erlangen (Franz-Penzoldt-Center). The animal procedures have been approved by the "Regierung of Unterfranken" (Approval Numbers: TS-9/2022 from January 1st 2022 and TS-4/2025 from May 1st 2025) and they were conducted in accordance with the guidelines of Federation of European Laboratory Animal Science Associations (FELASA).

C-P14

Immunological impact of combined kinase inhibitors and hypofractionated radiotherapy on the three-dimensional spheroid co-culture of HNSCC and cytotoxic T cells

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Background In the clinical management of head and neck squamous cell carcinoma (HNSCC), human papilloma virus (HPV)-negative tumors pose a challenge due to their increased radioresistance. A promising strategy to overcome this resistance is the additional use of DNA damage repair inhibitors (DDRi). To gain deeper insights into physiological and immunological responses to such treatments, a three-dimensional (3D) spheroid culture of head and neck squamous cell carcinoma (HNSCC) cell lines was established. The tumor microenvironment was represented by co-cultivated cytotoxic T cells. This study aimed to investigate the immunomodulatory changes of those T cells after treatment of the 3D spheroids with RT + DDRi.
Materials and methods To form spheroids, HPV-negative and HPV-positive HNSCC cell lines were seeded in *low-attachment U-bottom* plates, forming stable 3D structures within 24 h. These spheroids were treated with 1 µM AZD0156 (ATM inhibitor) or 0.1 µM VE-822 (ATR inhibitor), either alone or in combination with hypofractionated irradiation (2×5 Gy). In parallel, CD8 + T cells were isolated from peripheral blood using MACS beads, labeled with the live dye CFSE, and activated for 48 h in a CD3/CD28-coated plate. Flow cytometry was used to analyze the proliferation and expression of the activation markers CD69, CD25, HLA-DR and the immune checkpoint molecule PD-1 on the T cells 48 h after co-cultivation.
Results The periodically decreasing CFSE signal revealed three distinct T cell populations characterized by different proliferation rates. Initial results indicate that tumor spheroids treated with DDRi reduce the proliferation of T cells and a higher proportion of non-proliferating, CD25-expressing T cellscan be measured. HLA-DR and CD69, on the other hand, show a more dynamic regulation, which can manifest itself in both up- and down-regulation depending on the treatment modality.
Conclusions Treatment of tumor cell spheroids with DDRis and hypofractionated RT leads to immunomodulation and affects the proliferation and activity of CD8 + T cells in the 3D co-culture setup. The expression of the activation markers shows a correlation with cell proliferation. CD25 is detectable as an early marker, while HLA-DR is increasingly expressed later on proliferating cells.

Session D: Innovative approaches in radiotherapy

a) Key note lecture

D-K1

Tumor-to-draining lymph node communication is highly relevant for successful radioimmunotherapy

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Radiation Oncology 2025, **20(s1)**:D-K1

Background We previously demonstrated adjuvant lymph node irradiation as a promising approach to maximize radioimmunotherapy efficacy. We here investigated in detail the relevance and consequences of draining lymph node irradiation for combined radioimmunotherapy.
Materials and methods Using murine tumor models and naïve mice we investigated in detail irradiation-induced functional and structural changes in the lymph node to improve our understanding of the communication between the irradiated tumor and the draining lymph node. In vivo irradiated lymph nodes of C57BL/6 mice were analyzed for multiplex cytokine assessment, flow cytometry-based immunophenotyping, and immunohistochemical staining. In vitro experiments with primary lymph node-derived stromal cells were performed to complement in vivo and ex vivo performed experiments.

Results Longitudinal chemokine analysis of lymph nodes, which were irradiated with increasing doses of IR, revealed a highly specific and dose dependent decrease of the cytokines CCL19 and CCL21. These cytokines are of relevance for successful draining lymph node-to-tumor communication. Interestingly, cytokines that promote or limit T cell expansion and differentiation in the complex lymphoid environment were less affected. Concurrently, immunophenotyping of irradiated lymph nodes showed dose- and time-dependent lymphopenia in the lymph node, which also recovered over time in a dose-dependent manner, but only if irradiated below a sterilizing dose threshold. Structural changes within the irradiated lymph node point towards a a disrupted fibroblastic reticular cell network, loss of B cell follicle structure, and signs of fibrotic remodelling. In vitro experiments performed with lymph node-derived stromal cells allowed to differentiate between an IR-deregulated T-cell-to-stromal cell communication and direct IR-induced processes in stromal cells.
Conclusions Our findings indicate that the functional and structural integrity of the draining lymph node are pivotal for an intact CCR7-CCL19/CCL21 axis, which is required for an intact cancer immunity cycle. We will discuss how clinical protocols should be adapted to further exploit the potency of current combined radioimmunotherapy regimens.

b) Proffered papers—Oral presentation

D-O1

Adding the PPARα agonist enhances T cell-mediated effects of RT in combination with anti-PD-1

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Background RT and αPD-1 combination result in enhanced efficacy in both local and systemic (abscopal) tumor control, relying on CD8 + T cells. Many patients, however, do not respond. Fenofibrate (FFB), a PPARα agonist, approved for hypercholesterolemia and hypertriglyceridemia management, has previously shown antitumoral effects in mice models. Our study explores the antitumor effects of a triple combination comprising RT, αPD-1 and FFB in augmenting both local and abscopal tumor control in both abscopal tumor models.
Materials and methods Mice harboring bilateral B16 melanoma or C51 colon carcinoma tumors were treated with 8 Gy × 3 to the primary tumor. αPD-1 was administered weekly, and FFB was administered daily on weekdays for several weeks. Tumor volumes and overall survival were monitored. Tumor-specific CD8 T cells, tumor-associated high endothelial venules (TA-HEVs), and metabolic alterations in CD8 + T cells and tumor cells were assessed through flow cytometry (FACS).
Results The therapeutic efficacy of triple combination significantly surpassed that of all double combinations ($n \geq 6$ mice/group, $p < 0.05$) in both tumor models. Moreover, depletion of CD8 + T cells using anti-CD8 antibodies abrogated the enhanced tumor control and survival observed with the triple combination, rendering outcomes comparable to the double combinations. In line with these findings, we observed increased numbers of tumor-specific CD8 + T cells in both primary and secondary tumors receiving triple combination therapy ($n \geq 5$ mice/group: $p < 0.05$ compared to all respective double-treated groups). The triple combination group also lead to a significant increase in TA-HEVs ($n \geq 5$ mice/group: $p < 0.05$ compared to all respective double-treated groups). Furthermore, FFB treatment altered glycolysis and fatty acid oxidation in CD8 + T cells from both irradiated and non-irradiated tumor.
Conclusions Adding FFB to hRT + αPD-1 substantially enhanced the control of both primary and secondary tumors in a CD8 + T cell-dependent manner. These effects were associated with increased TA-HEVs, which promote T cell infiltration, as well as metabolic changes that may contribute to improved tumor control.

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D-O2

PD-L1/PD1 expression patterns define distinct tumor immune microenvironment subgroups in HNSCC in association with chemoradiation response

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Background Radio(chemo)therapy remains a cornerstone treatment for head and neck squamous cell carcinoma (HNSCC), yet therapeutic resistance and variable patient outcomes highlight the need for a deeper biological understanding. While immune checkpoint inhibition (ICI) targeting the PD-L1/PD1 axis is an emerging additional option, clinical trials have shown discrepant results regarding its efficacy in HNSCC. In the present study, we analyzed PD-L1/PD1 expression patterns, molecular classifiers, composition of the tumor microenvironment, and their relevance for clinical outcome following adjuvant radio(chemo)therapy to identify key determinants of treatment response and resistance.
Materials and methods Two retrospective HNSCC cohorts ($n = 113$, $n = 117$) treated with adjuvant radio(chemo)therapy at LMU-KKG were analyzed. PD-L1 positivity was assessed by immunohistochemistry, and polynomial risk modeling was applied to HPV-negative cases to associate PD-L1/PD1 expression with clinical outcomes. Risk groups (RGs) were compared for CD8-based immunotypes, molecular classifiers, and PD-L1/PD1 gene expression. Tumor microenvironment composition was further explored via bulk RNA-seq deconvolution and scRNA-seq analysis.
Results Polynomial risk modeling identified four risk groups (RG1-RG4) associated with tumor size, immunotypes, molecular classifiers, and PD-L1/PD1 expression. The high-risk group (RG3) displayed enrichment of immunosuppressed phenotype, elevated immune exhaustion markers, and co-expression of PD-L1/PD1, primarily from immune cells. In contrast, PD-L1 mono-expressors ($PD\text{-}L1^{hi}/PD1^{lo}$) exhibited tumor-specific PD-L1 expression and a lack of PD1 + T cells, potentially resistant to immune checkpoint inhibition (ICI) therapy. Myeloid cell enrichment and C9/C10 carcinoma ecotypes were predominant in $PD\text{-}L1^{hi}/PD1^{hi}$ tumors with immune-exhausted phenotypes.
Conclusions Polynomial risk modeling based on PD-L1 expression delineated four PD-L1-based risk groups in HNSCC undergoing adjuvant radio(chemo)therapy. Distinct PD-L1/PD1 co-expression patterns reflect cell type-specific PD-L1/PD1 expression. A $PD\text{-}L1^{hi}/PD1^{hi}$, myeloid cell-enriched subgroup of patients with elevated immune exhaustion features was identified which presumably may respond to ICI therapy. Conversely, $PD\text{-}L1^{hi}/PD1^{lo}$ expressing tumors, lacking PD1 expressing T cells, most likely represent an ICI-resistant subgroup.
Acknowledgements This study was supported by the Bundesministerium für Bildung und Forschung (BMBF, 02NUK086B and 02NUK087).
Trial registration and/or ethics approval number The study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the LMU's ethics committee (EA 312-12, 448-13, 17-116).

D-O3

Defining predictive immune markers by immune monitoring and machine learning from the blood of HNSCC patients in the prospective DIREKHT trial

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Radiation Oncology 2025, **20(s1)**:D-O3

Background In the staging and treatment of distinct solid tumor entities, immunological biomarkers gain more and more importance. However, in the treatment of head and neck squamous cell carcinoma (HNSCC), such biomarkers, particularly those derived from peripheral blood, have hardly been investigated. In order to address this issue, the prospective DIREKHT study (NCT02528955) integrated a translational research program in which the longitudinal immune status of patients was monitored throughout their treatment. The aim was to identify predictive immune signatures for prognosis and therapy optimization for HNSCC.

Materials and methods The immune status of HNSCC patients (n = 70, including 21 with oral cavity, 48 with oropharyngeal tumors and 1 with laryngeal tumor) was assessed before and after radio-(chemo)therapy (R(C)T), as well as during follow-up. A detailed flow cytometry-based assay was used to analyze 45 different immune parameters from the patients' whole blood. The data was utilized to train a machine learning model that predicts disease-free survival achieving MCC = 0.63. The training process involved a data-based variable selection, followed by the development of a logistic regression model. The model's validity was verified through nested cross-validation using multiple repeats of stratified K-fold cross-validation in the inner and outer loop.

Results By evaluating the immune status before and after R(C)T, a signature of various immune parameters was identified that could predict disease-free survival in patients. Key components of this signature included the HLA-DR expression on T cells and monocytes after RT, pre-treatment frequencies of basophils and natural killer cells, and the frequency of HLA-DR-positive monocytes before RT. Notably, the stability and predictive accuracy of this immune signature were independent of clinical factors such as human papilloma infection or tumor location. Thus, including these clinical parameters did not further enhance the predictive power of the immune signature.

Conclusions These findings highlight the importance of analyzing the peripheral immune status in HNSCC patients for identifying immunological biomarkers, which could potentially allow for more individualized therapy. The immune signature presented here can predict disease-free survival in HNSCC patients undergoing R(C)T. In the future this signature needs to be validated in a larger patient cohort to make it accessible for clinical use.

Trial registration and/or ethics approval number The study protocol was approved by the local ethics committee No.195_14B and registered in clinicaltrials.gov NCT02528955.

D-O4

Recruitment of myeloid immune cell populations upon IORT in breast cancer patients is mediated by normal tissue-derived cytokines

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Radiation Oncology 2025, **20(s1)**:D-O4

Background Radiotherapy plays a central role in the management of breast cancer, especially following breast-conserving surgery. Treatment options include a range of radiation schedules, from (hypo-)fractionated regimens to high-dose ablative approaches. Intraoperative radiotherapy (IORT) delivers a concentrated dose of radiation (typically 20 Gy) directly to the tumor bed during surgery, allowing for targeted treatment of residual tumor cells in the surrounding tissue. Beyond its cytotoxic effects, radiotherapy has been shown in preclinical models to activate both innate and adaptive immune responses, which may contribute significantly to tumor control. A critical aspect of this response involves the recruitment of immune cells to the irradiated site. To investigate these mechanisms in a clinical setting, we conducted a translational study analyzing wound fluid and peripheral blood samples from breast cancer patients undergoing breast-conserving surgery, with or without IORT. The study focused on cellular changes and molecular characteristics associated with immune activation.

Materials and methods Wound fluids were assessed for volume, hemoglobin content, total leukocyte counts, and specific leukocyte subpopulations. Cytokine profiles and transcriptomic changes were also analyzed. In parallel, in vitro studies were performed on non-malignant cells of the breast to examine their response to 20 Gy irradiation.

Results IORT was associated with increased infiltration of three myeloid immune cell populations into the tumor bed, as well as transient elevations in immune cell subsets in peripheral blood. Analysis of wound fluids from IORT patients revealed significantly higher levels of two distinct cytokines, which correlated with the presence of specific immune cell populations, suggesting a role of these cytokines in immune cell recruitment. In vitro irradiation of non-malignant breast cells also led to a robust senescence response and enhanced secretion of these cytokines, indicating that normal tissue contributes actively to post-irradiation immune signaling.

Conclusions This study sheds light on the immunological impact of high-dose IORT in breast cancer treatment. It demonstrates both local and systemic immune responses, characterized by specific cytokine patterns and immune cell recruitment, and highlights the role of non-malignant tissue in mediating these effects. These findings provide a foundation for future research aimed at harnessing the immunomodulatory potential of single high-dose radiation therapies to improve clinical outcomes.

Trial registration and/or ethics approval number Clinical Trials 246-15 and 20-488 of University Hospital, LMU Munich, Munich Germany.

c) Proffered papers—Poster presentation

D-P1

Integrating Digital Cytometry for tumor microenvironment profiling and prognostic insights in metabolic subtypes of HNSCC

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Radiation Oncology 2025, **20(s1)**:D-P1

Background Head and Neck Squamous Cell Carcinoma (HNSCC) is a heterogeneous malignancy affecting the oral cavity, pharynx, and larynx. Although radio(chemo)therapy remains the primary therapeutic approach, treatment resistance and high variability in patient response underscore the need for further investigation. Previous findings from our group [1] indicate that HNSCC can be classified into metabolic pathway-based subtypes (MPS), each exhibiting distinct adjuvant treatment responses. Notably, MPS1 is a high-risk subtype, characterized by upregulated tumor processes, including tumor microenvironment (TME) remodeling, cancer-associated fibroblast (CAF) activation, and immune suppression. A deeper understanding of MPS-specific TME composition, underlying processes, and their impact on therapeutic radiation response is crucial for identifying potential prognostic biomarkers and therapeutic targets.
Materials and methods Digital cytometry algorithms enable the in silico extraction of cell type proportions and gene expression profiles from tumor bulk RNAseq data, providing valuable insights into the TME. Additionally, these methods facilitate cell type-specific analysis in large tumor transcriptomic datasets and enable associations with clinical follow-up data. However, the wide range of available tools and the absence of a standardized benchmarking procedure make selecting the most suitable tool for a given research context challenging. To apply digital cytometry in translational radiation oncology, this study employs a two-fold approach: (i) Technical assessment of CIBERSORTx, BayesPrism and InstaPrism using RNAseq profiles from pre-defined in vitro cell mixtures. (ii) In-depth characterization of MPS TME organization, immuno-metabolic processes, and cell interactions through the integration of tumor bulk, single-cell, and spatial transcriptomic data.
Results These findings are expected to demonstrate that our approach enables a robust and reliable evaluation of digital cytometry methods, facilitating the selection of the most suitable tool. The subsequent comprehensive data analysis will provide key insights into metabolic subtypes in HNSCC, elucidating their association with patient clinical outcomes following adjuvant tumor therapy.
Conclusions This study will advance the integration of digital cytometry and multi-level data analysis into translational radiation oncology, contributing to a deeper understanding of critical biological processes within the TME and their influence on adjuvant therapy response in HNSCC.
Trial registration and/or ethics approval number Ethical approval (EA) for this study was obtained by the ethics committee of the LMU (EA 312-12, 448-13, 17-116).
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D-P2 Tuning metabolism via SLC25A1 inhibition improves chemotherapy response in combination with radiotherapy in HNSCC cell lines

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Radiation Oncology 2025, **20(s1)**:D-P2

Background Head and neck squamous cell carcinoma (HNSCC) exhibits distinct metabolic phenotypes that are associated with varying degrees of radiosensitivity. Recent own work demonstrated that inhibition of the citrate carrier SLC25A1 can lead to a pharmacologically induced defect in homologous recombination repair (HRR), increasing radiosensitivity in lung and glioblastoma cell lines [1, 2]. We hypothesized that inhibiting SLC25A1 in HNSCC using a small molecule inhibitor CTPI-2 could similarly lead to radiosensitization by inducing the accumulation of D-2-hydroxyglutarate (D-2HG), which in turn could inhibit DNA repair enzymes responsible for alkylating DNA damage. This strategy aimed not only to impair the DNA repair machinery but also to sensitize HNSCC cells to alkylating agents such as cisplatin, temozolomide and the antimetabolite 5-fluorouracil (5-FU) used as chemotherapeutics in the treatment of HNSCC.
Materials and methods We performed time-resolved metabolic (Seahorse Bioanalyzer), radiobiological (ROS, cell death, apoptosis levels and cell proliferation) and long-term survival assays in a panel of HNSCC cell lines. We combined irradiation (2-10 Gy) with inhibition of mitochondrial citrate transport (CTPI-2) to impair mitochondrial function and DNA repair mechanisms and subsequent combination with a clinically relevant chemotherapeutics (cisplatin, temozolomide, 5-FU) to improve the sensitivity to irradiation. Proof-of-concept experiments were performed using 3D Spheroids and the chicken allantoic membrane (CAM) model.
Results Sub-lethal doses of CTPI-2, either alone or in combination with sub-lethal doses of chemotherapeutics and irradiation, effectively inhibited proliferation and long-term survival of HNSCC cell lines. Additionally, treatment led to a significant reduction in 3D spheroid size and tumor growth in the in vivo CAM model. In contrast, preliminary experiments on normal tissue cell lines indicated that sub-lethal concentrations of CTPI-2 and chemotherapeutics had no significant impact on the long-term survival of non-cancerous cells.
Conclusions The combination of CTPI-2 with clinically relevant chemotherapeutics further enhanced the radiosensitization of HNSCC cell lines, highlighting a promising therapeutic approach to improving treatment efficacy through metabolic modulation.
Acknowledgements Supported by grant of BMBF (02NUK061B).

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D-P3 Longitudinal monitoring of laboratory markers and immune status during short term Dance Movement Therapy for radio-oncological patients (Dance-RT-01-Trial)

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Radiation Oncology 2025, **20(s1)**:D-P3

Background Integrative Dance Movement Therapy (DMT) has shown potential benefits for physical and psychological health in patients with

somatic diseases, yet objective evidence, particularly for immunological effects in radio-oncological patients, is lacking. Thus, the feasibility Dance-RT-01 (Ethics Committee of the University Hospital Erlangen Reference Number: 24-505-B) trial addresses this issue by focusing on immunophenotyping to investigate whether DMT applied shortly after radiotherapy (RT) can induce positive measurable changes in immune parameters. The study further explores the clinical benefits regarding functional capacity, reduction of fatigue and improvement of quality of life (QoL).

Materials and methods The study is designed as investigator-initiated, prospective, non-randomized feasibility trial and includes radio-oncological patients with breast or prostate cancer (ECOG 0-2) who recently completed RT. Participants complete five weekly DMT sessions (90 min each) combined with psychotherapeutic elements. The therapy is carried out by one specially trained therapist "Leiterin für therapeutischen Tanz, DGT" with experience in radio-oncology. Recruitment starts in February 2025. Detailed immunophenotyping (longitudinal analysis for detailed immune status over the whole therapy) is performed using advanced multicolor flow cytometry to analyze immune cell subsets (e.g., T cells, NK cells, monocytes), their activation states, and shifts in pro- and anti-inflammatory mediators. Additional laboratory analysis covers inflammatory markers (e.g., CRP, gamma-GT), hormone levels (e.g., cortisol, TSH), and nutritional biomarkers (e.g., vitamin D, B12, folic acid). Secondary endpoints include assessments of body composition via Body Impedance Analysis (BIA), functional capacity (maximum grip strength with Jamar hand dynamometer), and QoL using the standardized and validated questionnaire EQ-D5-5L (European Quality of Life 5 Dimensions 5 Level Version).

Results Early results, expected in Q2/2025, will evaluate correlations between immunological changes, inflammatory markers, clinical improvements, and patient-reported outcomes (PROs).

Conclusions Dance-RT-01 aims to provide first hints on physiological and psychological effects of DMT as complementary therapy method for tumor patients. It is the first prospective feasibility study and is the basis for future studies proving benefits of DMT after radiotherapy.

Trial registration and/or ethics approval number (if applicable) Ethics Committee of the University Hospital Erlangen Reference Number: 24-505-B.

D-P4

Integrative multi-omics analysis of metabolic subtypes in HNSCC: insights into tumor microenvironment interactions and adjuvant therapy response

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Radiation Oncology 2025, **20(s1)**:D-P4

Background Radiochemotherapy remains a primary therapeutic approach for head and neck squamous cell carcinoma (HNSCC). However, wide variations in patient responses, driven by treatment resistance, highlight the urgent need for a deeper characterization of the underlying tumor biology. Our group previously identified metabolic pathway-based subtypes (MPS) in HNSCC, which exhibit distinct responses to adjuvant treatment. Notably, the high-risk MPS1 subtype is associated with elevated tumor-promoting processes, including tumor microenvironment (TME) remodeling, increased prevalence of cancer-associated fibroblasts (CAFs), and immune suppression.

Materials and methods This study aims to further elucidate the metabolic properties of MPSs in HNSCC through an integrative approach combining RNAseq-based transcriptomics and FT-ICR mass spectrometry-based untargeted metabolomics, using paired normal and tumor tissue samples from an adjuvant clinical cohort. To achieve this, we construct bipartite networks, where nodes represent multi-omics entities and edges are derived from pathway databases such as KEGG. Additionally, integrative multivariate analytical methods, such as multi-omics factor analysis (MOFA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA), are applied to identify underlying patterns in the combined datasets. Furthermore, using in vitro model systems consisting of MPS-classified HNSCC cell lines and native primary fibroblasts, we will investigate their interactions and potential CAF polarization by integrating transcriptomic and metabolomic data, along with multiple cytometric endpoints.

Results Integrating transcriptomic and metabolomic data is expected to uncover distinct metabolic features associated with MPS in HNSCC, offering deeper insights beyond those obtained from transcriptomic data alone. Multivariate analyses will likely identify additional key molecular patterns that distinguish low- and high-risk MPS tumors, while network-based analysis will help bridge missing links between metabolic clusters, advancing drug target identification. In vitro co-culture experiments are anticipated to provide valuable insights into tumor cell–fibroblast interactions via cytokine signaling. Differential fibroblast polarization and evidence of CAF polarization induced by high-risk MPS1 tumor cells are expected, enabling the identification of key signaling molecules. The integration of cytometric, metabolomic, and transcriptomic analyses will offer a comprehensive molecular characterization of MPS-specific fibroblast phenotypes. Overall, these findings will enhance our understanding of metabolic rewiring in HNSCC and its impact on tumor progression and therapy response.

Conclusions This integrative multi-omics approach enables a comprehensive characterization of metabolic processes in HNSCC tumors and adjacent normal tissues, providing critical insights into tumor metabolism and its influence on adjuvant therapy response. In vitro co-cultivation experiments will further elucidate the cross-talk between malignant cells and fibroblasts within the HNSCC tumor microenvironment (TME), contributing to the identification of key biological processes and molecular targets that may enhance the radiotherapeutic response in high-risk HNSCC tumors. A deeper understanding of malignant cell–fibroblast interactions is crucial for the development of targeted, multimodal tumor therapy strategies.

Trial registration and/or ethics approval number Ethical approval (EA) for this study was obtained by the ethics committee of the LMU (EA 312-12, 448-13, 17-116).

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D-P5

Validation of the prognostic significance of a 5-miRNA signature in HPV-negative head and neck cancer after adjuvant radio(chemo) therapy

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Radiation Oncology 2025, **20(s1)**:D-P5

Background In an initial study, we identified a 5-microRNA (miRNA) signature that was prognostic for freedom from recurrence (FFR) in a retrospective multicentre cohort of HPV-negative, locally advanced head and neck squamous cell carcinoma (HNSCC) patients treated with adjuvant radiochemotherapy and validated it in a monocentric HPV-negative HNSCC cohort of patients treated with adjuvant radio(chemo)

therapy. The model was independent of extracapsular extension (ECE), TNM T stage and lymphovascular invasion (LVI) [1]. In the present study, the prognostic significance of the 5-miRNA signature was analysed in a prospectively collected HNSCC collective.
Materials and methods 89 patients with locally advanced HPV-negative HNSCC treated with adjuvant radio(chemo)therapy at the LMU Department of Radiation Oncology were included. Simultaneous DNA/RNA isolation was performed from histomorphologically verified formalin-fixed paraffin-embedded tissue sections after macrodissection. Global miRNA expression analysis was performed using Agilent miRNA microarrays. Risk score calculation and the dichotomisation into high/low risk were performed using the coefficients and the cut-off from the risk model of the discovery study. The risk groups were analysed in a univariate and multivariable Cox proportional hazard analysis with regard to overall survival (OS) and freedom from recurrence (locoregional and/or distant failure).
Results From the 89 patients, n = 51 were assigned to the 5-miRNA high-risk group and n = 38 to the low-risk group. The 5-miRNA risk groups significantly predicted FFR ($p = 0.019$, HR: 3.11, 95%-CI 1.15–8.46) in univariate analysis and in a multivariable model together with ECE ($p = 0.025$, HR: 3.52, 95%-CI 1.18–10.52). A Cox model combining the 5-miRNA risk with ECE significantly predicted FFR, while patients were significantly split into 5-miRNA risk groups. Univariate and multivariable Cox analysis for OS showed qualitatively similar results.
Conclusions The 5-miRNA signature predicts freedom from recurrence after multimodal therapy in locally advanced HNSCC. Similar to the retrospective discovery study, the 5-miRNA signature split ECE-negative patients. The 5-miRNA signature was positively validated in an independent HNSCC cohort and thus gains potential for clinical application.
Trial registration and/or ethics approval number Ethical approval (EA) for this study was obtained by the ethics committee of the LMU (EA 312-12, 448-13, 17-116).

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D-P6

HPV-negative HNSCC cell lines can be radiosensitized by the MRN inhibitor mirin

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Radiation Oncology 2025, **20(s1)**:D-P6

Background Intrinsic chemo- and radioresistance of human papilloma virus (HPV) -negative head and neck squamous cell carcinoma (HNSCC) result in a poor prognosis for patients. Combining ionizing radiation (IR) with small molecule inhibitors (SMI) of the cellular DNA repair mechanisms has radiosensitizing potential.
Materials and methods We used the MRN (Mre11, Rad50, Nbs1) inhibitor Mirin (30 µM) to treat five HPV-negative and two HPV-positive HNSCC cell lines. Fibroblasts served as healthy control. The cells additionally received 2 Gray IR 3 h after Mirin addition. We determined apoptosis (Annexin V), necrosis (7-AAD), and cell cycle distribution (Hoechst) with flow cytometry. Moreover, we measured DNA damage (γH2AX) with immunostaining and colony formation.
Results Colony formation of HPV-negative cell lines was clearly reduced by Mirin (surviving fraction (SF) 0.13–0.49 normalized to DMSO control). Moreover, they were radiosensitized (SF 0.08–0.34) by the combined treatment, whereas the HPV-positive cell lines did not profit from Mirin or the combined treatment at all. The increase of cells in G2/M phase from IR alone to the combined treatment by 6.4–18.5% also resulted from the radiosensitizing effect of Mirin in HPV-negative HNSCC cell lines. In contrast, the amount of HPV-positive cells in G2/M phase after IR and the combined treatment was comparable. Regarding cell death, HPV-positive cell lines were not sensitive to Mirin as well but in HPV-negative ones the number of dead cells increased by 9.8–23.4% after the combined treatment compared to IR alone.
Conclusions Inhibition of the MRN complex by the SMI Mirin led to radiosensitization of all tested HPV-negative HNSCC cell lines. This general effect has never been observed with any other inhibitor in our group; instead, in other studies there were always cell line dependent effects. In contrast, HPV-positive cell lines did not react to Mirin or the combined treatment with IR. In the future, the different underlying mechanisms in HPV-positive and -negative HNSCCs should be investigated in detail. Nevertheless, Mirin in combination with IR is a promising molecule for the treatment of therapy-resistant HPV-negative HNSCCs.

D-P7

Nanoparticle-based delivery of therapeutic siRNAs targeting Survivin in NSCLC cell lines

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Radiation Oncology 2025, **20(s1)**:D-P7

Background Survivin, a key member of the inhibitor of apoptosis protein (IAP) family, plays a crucial role in tumor cell survival and resistance to radiotherapy. Targeting Survivin using siRNA offers a potential strategy to enhance radiosensitivity, but efficient intracellular delivery remains a challenge due to siRNA's inherent instability and poor cellular uptake. This study evaluates two nanoparticle-based delivery platforms designed to enhance Survivin knockdown and improve therapeutic efficacy in non-small cell lung cancer (NSCLC) models.
Materials and methods Human NSCLC (H23, H1299) lines and murine LL2 cells were transfected with Survivin-specific siRNAs using two different nanoparticle formulations: tyrosine-modified linear polyethyleneimine (LP10Y) and polypropylene dendrimers (PPI-Y). Roti-Fect PLUS served as a reference control. Western blotting was performed at 24 h (murine cells) and 48 h (human cells) post-transfection to assess Survivin expression. Following transfection, cells were irradiated at doses of 2, 4, or 6 Gy (X-ray, single exposure). Apoptosis rates were quantified via cytofluorometry, and the ability of cells to survive and form colonies was evaluated in a 3D environment using a laminin-rich extracellular matrix. DNA damage repair capacity was determined by analyzing γH2AX/53BP1 foci by microscopic quantification.
Results Both nanoparticle formulations significantly enhanced siRNA delivery efficiency and Survivin knockdown compared to Roti-Fect PLUS across all tested cell lines. Among the nanoparticle-based systems, PPI-Y achieved the highest level of Survivin suppression. Functional assays revealed that cells treated with PPI-Y nanoparticles demonstrated the most potent radiosensitizing effect while maintaining the lowest toxicity. Moreover, the inhibition of DNA repair, as evidenced by an increase in persistent γH2AX/53BP1 foci, was most pronounced in PPI-Y-treated LL2 cells, while LP10Y and Roti-Fect PLUS exhibited moderate effects.
Conclusions The use of nanoparticles for siRNA-mediated attenuation of Survivin significantly enhances therapeutic efficacy in NSCLC models. PPI-Y, in particular, demonstrates superior delivery efficiency and radiosensitizing potential, making it a promising candidate for further preclinical evaluation in an orthotopic LL2 tumor model.
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D-P8

Exploiting metabolic vulnerabilities for radiosensitization in medulloblastoma

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Background Medulloblastoma (MB), the most common malignant brain tumor in children, is classified into molecular subgroups with distinct prognoses and therapeutic responses. These subgroups include WNT-MB, SHH-MB TP53 wild type, SHH-MB TP53 mutated, and non-WNT/non-SHH, with further epigenetic divisions into group 3 (MB-G3) and group 4 (MB-G4). While WNT-MB is associated with favorable outcomes, MYC-amplified MB-G3 and SHH-MB TP53 mutated subtypes have poor progression-free survival rates (< 20%). Current treatments, including radiation, often cause severe long-term side effects. Our goal is to identify novel metabolic targets for radiosensitization of MB, alone or in combination with PARP1/2 inhibition, to improve therapeutic efficacy and reduce adverse effects.
Materials and methods We performed a basal radiobiological characterization of MB cell lines representing the SHH-MB (DAOY, ONS-76) or MB-G3/4 (D283, HDMB03) subgroups. Therefore, we analyzed short-term (24–72 h proliferation, cell death) and long-term effects (colony formation assay) upon irradiation (0–10 Gy). Additionally, we have performed functional metabolic characterization of aforementioned cell lines upon IR and combination with PARP-inhibitors (pamiparib, olaparib) by using Seahorse XFe96 Bioanalyzer (Agilent) and metabolic phenotyping by using ODIN-L (Biolog). We plan to validate our findings *in-ovo* by using a chicken chorioallantoic membrane (CAM) model.
Results Our first results to increased short-term proliferation ability of radioresistant SHH-MB cell lines as well as increased metabolic activity and total ATP levels compared to radiosensitive MB-G3/4 cell lines. Changes in metabolic phenotypes upon irradiation alone or in combination with Pamiparib are currently ongoing.
Conclusions Our findings revealed distinct metabolic profiles and radiobiological characteristics between SHH-MB and MB-G3/4 subgroups, with SHH-MB cell lines showing increased proliferation and metabolic activity compared to MB-G3/4. These preliminary results suggest that targeting metabolic pathways, particularly in combination with PARP inhibition, may provide a promising approach to radiosensitize resistant MB subtypes. Ongoing studies, including functional metabolic analyses and validation in the CAM model, aim to further elucidate therapeutic vulnerabilities and pave the way for novel, subtype-specific treatment strategies.
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D-P9

Tumor microenvironment subtypes of HPV-negative head and neck squamous cell carcinoma reconstructed from bulk transcriptome profiles

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Background The tumor microenvironment (TME) influences progression and treatment response in head and neck squamous cell carcinoma (HNSCC). While molecular subtypes have been extensively studied, TME heterogeneity in HPV (human papillomavirus)-negative HNSCC remains underexplored. We aimed to identify transcriptome-derived TME subtypes (hTMEs) and evaluate their clinical and biological significance.
Materials and methods To identify hTMEs we applied the EcoTyper framework(1) to transcriptomes from HPV-negative TCGA-HNSC(2, 3) cases (n = 242), using a single-cell HNSCC reference. Subtypes were reconstructed in independent HPV-negative cohorts (LMU-KKG(4), DKTK-ROG(5), LIFE-HNG(6); n = 469) and validated by spatial transcriptomics. Associations with cell states, clinical parameters and survival outcomes (overall-/recurrence-free survival, freedom from recurrence) were assessed using Cox models. Additionally, we calculated the radiosensitivity index(7) (RSI) and genomic-adjusted radiation dose(8) (GARD) for LMU-KKG, DKTK-ROG and analyzed their associations with hTMEs, cell states, and clinical variables.
Results Four hTMEs were identified with distinct immune and stromal compositions. hTME-1 and hTME-2 were associated with favorable prognosis, while hTME-3 showed reduced OS (HR 1.68) and RFS (HR 1.74) independent of clinical prognosticators TNM-N, extracapsular spread and lymphovascular invasion. Spatial transcriptomics confirmed spatially colocalized enrichment of p-EMT-like malignant cells and CAF-rich fibroblasts in hTME-3. hTMEs correlated with tumor localization, immune signatures and molecular classifications, but not with TNM stage. hTME-1 and hTME-3 showed increased RSI and decreased GARD compared to hTME-2 ($p < 0.05$), indicating higher intrinsic radioresistance and reduced biological effectiveness of the applied radiation doses (mean RSI/GARD/total dose: hTME-1, 0.22/52.2 Gy/64.1 Gy; hTME-3, 0.27/51.3 Gy/64.5 Gy; hTME-2, 0.17/64.5 Gy/64.3 Gy).
Conclusions hTMEs delineate biologically and clinically distinct ecosystems in HPV-negative HNSCC while hTME-3 was independently associated with poor prognosis and characterized by spatially colocalized p-EMT-like malignant cells and CAF-rich fibroblasts. Via RSI and GARD, hTMEs were linked to intrinsic radiosensitivity and biological efficacy of delivered doses and provide potential in stratified radiotherapy in HPV-negative HNSCC.
Ethics approval numbers EA 312-12, 448-13, 17-116.

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D-P10

Prognostic value of Dicer and CA9 expression in patients with anal squamous cell carcinoma treated with chemoradiotherapy

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Background The endoribonuclease Dicer covers an enzyme enabling cleavage of double-stranded pre-microRNA into short double-stranded RNA fragments (microRNA) while Carbonic anhydrase 9 (CA9) is a transmembrane dimeric metalloenzyme that facilitates acid secretion. An inverse correlation between the two markers was reported for example in hepatocellular cancer. The aim of the study was to evaluate the expression and a prognostic value of biomarker DICER and CA9 expression in patients treated with definitive chemoradiotherapy (CRT) for anal squamous cell carcinoma.
Materials and methods The biomarkers were scored by multiplex immunofluorescence (Vectra 3 quantitative pathology imaging system, Perkin Elmer, n = 92) on pre-treatment biopsies and correlated with patient´s histopathological characteristics and clinical endpoints cumulative incidence of local control (LC), distant recurrences (DC), disease-free survival (DFS) and overall survival (OS).
Results We observed a significant correlation between DICER and CA9 expression. By contrast, DICER and CA9 expression does not correlate with age, gender T/NM stage and grading. High levels of DICER detection (> median) were predictive for improved LC ($p = 0.012$) and DFS ($p = 0.048$), while elevated levels of CA9 (> median) correlated with decreased LC ($p = 0.009$), decreased DC ($p = 0.001$), decreased DFS ($p = 0.022$) and OS ($p = 0.008$). In multivariate analyses, Dicer and CA9 detection remained significant for LC ($p = 0.009$, and $p = 0.028$, respectively).
Conclusions These data indicate that elevated levels of pretreatment CA9 and low levels of DICER are correlated with an unfavourable clinical outcome in patients with anal carcinoma treated with definitive CRT. These retrospective data are currently being validated in a prospective RADIANCE clinical trial.
Trial registration and/or ethics approval number Ethical approval (EA) for this study was obtained by the ethics committee of the University Hospital of Frankfurt am Main (Protocol Number 458/17).

D-P11

Multimodal prediction of clinical and molecular features in adjuvantly radio(chemo)therapy treated locally advanced HPV-negative head and neck squamous cell carcinoma

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Background Locally advanced HPV (human paillomavirus)-negative head and neck squamous cell carcinoma (HNSCC) presents with substantial molecular and clinical heterogeneity, contributing to poor prognosis despite multimodal treatment. There is an unmet need for integrative biomarkers that leverage routine diagnostic modalities to support risk stratification and guide treatment decisions.
Materials and methods We analyzed three cohorts (TCGA [1], LMU-KKG [2]), DKTK-ROG[3]) of patients with locally advanced HPV-negative HNSCC treated with adjuvant radio(chemotherapy) for which transcriptomic data, digitized histopathology slides, and radiotherapy planning CTs were available. Deep learning models were developed to predict molecular subgroups and clinical parameters from histology alone, using attention mechanisms for representability. In parallel, convolutional neural networks were trained on planning CTs to predict clinical outcomes and variables. A multimodal framework was implemented to integrate transcriptomics, histology, and imaging data for joint prediction tasks.
Results Histology-based models accurately predicted transcriptome-defined molecular subgroups and key clinical variables. Attention maps highlighted morphological correlates of predictive features, supporting model transparency and paving the way for automated histological annotation. CT-based models achieved reliable performance in predicting outcome-related clinical variables. Importantly, multimodal integration of transcriptomic, histological, and imaging data led to improved predictive accuracy across endpoints compared to single-modality models.
Conclusions This study demonstrates the feasibility and added value of AI-based multimodal prediction in locally advanced HPV-negative HNSCC. By integrating routine histology, imaging and molecular profiles, we lay the foundation for interpretable and clinically applicable tools to support precision oncology in a high-risk patient population.
Trial registration and/or ethics approval number EA 312-12, 448-13, 17-116.

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D-P12

An innovative approach for identifying subgroups of breast cancer patients with different risk of fibrosis following radiotherapy

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Background The development of late toxicity after radiotherapy is thought to be influenced by multiple risk factors with modest effect sizes. In this study, the hypothesis was tested that some factors may only become relevant within distinct subgroups defined by different functional pathways [1], and the potential for predictive modeling was assessed.
Materials and methods DNA, CD4 + radiation-induced lymphocyte apoptosis (RILA) data, and long-term follow-up (10.3–12.8 years) were collected from n = 238 patients in the German ISE breast cancer cohort [2]. Moderate-severe fibrosis was observed in 71 patients (29.8%). Three candidate SNPs, which had been identified previously, were genotyped through PCR. Predictive modeling was carried out using partition analysis (PA) and ensemble machine learning (ML) methods. The identities of the two SNPs and the details of the ML models will be disclosed at the DeGBS meeting.

Results Significant associations with breast fibrosis were found for two SNPs and two clinical parameters (BMI and hypertension, HTN). Six subgroups were identified by PA using these features along with RILA values. It was observed that BMI and the two SNPs were significantly associated with fibrosis in the RILA-low subgroup (high-risk) but not in the RILA-high subgroup (low-risk). The opposite was detected for HTN, which showed significance only in the RILA-high subgroup. No significant associations were found within the complementary RILA subgroups, thereby providing strong support for our subgroup hypothesis. Three risk groups (low, intermediate, high) were formed from the six subgroups, showing a five-fold difference in fibrosis risk ($p < 0.0001$). Two ML models were created, which predictions were combined. Three risk groups were generated, showing a strong correlation with the risk groups identified by PA ($p < 0.0001$). Based on the five-feature ML model, 80% of patients were classified as either high-risk (13.7% with 83.3% fibrosis risk) or low-risk (66.7% with 17.1% fibrosis risk), and an AUC of 0.735 was achieved in ROC analysis.
Conclusions Strong support for our subgroup hypothesis and robustness of the combined ML model was demonstrated. However, external validation could not be performed due to the absence of other cohorts with long-term follow-up and CD4 + RILA data. A potential reduction of fibrosis in more than a third of patients could be achieved through the use of the five-feature ML model, by offering alternative treatment options, such as partial-breast radiotherapy, to high-risk patients. The correlation with PA suggests that mechanistic insights could be obtained, and further model improvements could be explored.
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Trial registration and/or ethics approval number Ethics approval number: 062/2002, Medical Faculty Heidelberg.

Declaration of interests AS, MRV and CH are co-inventors of a patent application submitted by Heidelberg University. All other authors declare no conflicting financial interests relating to this work.

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D-P13

Microbeam radiation therapy (MRT) and minibeam radiation therapy (MBRT) improve local tumor control in a mouse tumor study

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Radiation Oncology 2025, **20(s1)**:D-P13

Background Microbeam Radiation Therapy (MRT) and minibeam radiation therapy (MBRT) are two innovative preclinical concepts in radiotherapy that collimates X-ray radiation in micrometer-wide, planar beams. Previous research has shown that MRT substantially spares normal tissue, while being equally effective in tumor ablation. The aim of this study was to determine the tumor control probability in an in vivo mouse xenograft model comparing MRT and MBRT with conventional broadbeam radiotherapy (BB).
Materials and methods 8–12 week old immunocompromised CD-1 Foxn1nu mice from Charles River were injected subcutaneously into the right flank with A549 cells (human non-small cell lung carcinoma). Once the tumors reached a volume of ≥ 60 mm^3, the mice were randomly assigned to specific groups and irradiated accordingly. All animals were irradiated with X-rays using a self-developed setup within the small animal irradiation device, XenX by X-Strahl. Tumors were irradiated by MRT with microbeams of 100 µm in width, with a CTC (center to center distance) of about 416 µm and a PVDR of 25. Tumors were irradiated by MBRT with minibeams of 525 µm in width, with a CTC (center to center distance) of about 2100 µm and a PVDR of 24. The irradiation time for MRT and MBRT was determined using the concept of the equivalent uniform dose (EUD) based on the linear quadratic model (LQM). Tumor volumes after treatment were constantly measured with a caliper for a total follow-up time of 120 days. The volumes obtained were analyzed to determine tumor control or regrowth. The percentages of tumor control among all the dose groups for MRT, MBRT and BB were fitted with a logistic regression and the corresponding TCD50 values were obtained.
Results The TCD50 (dose needed to control 50% of the tumors) values obtained for MRT and MBRT were similar with TCD50 values of 20 ± 2 Gy and 21 ± 1 Gy, respectively. For animals irradiated with conventional broad beam irradiation (BB), the TCD50 value was significant higher (32 ± 3 Gy; $p < 0.001$).
Conclusions Our results demonstrate that both microbeams and minibeams perform significantly better in controlling tumors than conventional treatment. The decrease in dose broadens the therapeutic window of MRT and therefore reduces normal tissue side effects. This suggests a potential benefit of MRT and MBRT for the treatment of human lung cancer. Furthermore, we observed no significant differences between MRT and MBRT in terms of tumor control probability.
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D-P14

Molecular profiling of primary and radiogenic angiosarcoma

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Background Angiosarcomas (AS) are rare, aggressive malignancies arising from endothelial cells in various anatomical sites. They can occur as primary tumors or develop secondarily following radiotherapy. While key driver genes of AS have already been identified, specific molecular changes of radiogenic AS remain poorly characterized and the subject of ongoing debate.
Materials and methods We performed 3'RNA sequencing and array comparative genomic hybridization (aCGH) from formalin-fixed paraffin-embedded tissue sections of a single-center cohort (LMU University Hospital Munich) consisting of 15 sporadic and 12 radiogenic AS. Radiogenic AS in this study have developed in the breast of female patients who had previously undergone radiotherapy for breast cancer. The secondary tumors arose within the high-dose radiation field of previous radiotherapy. Our goal was to investigate molecular alterations in both AS subgroups to identify molecular markers associated with their different pathogenesis. We analyzed genome-wide copy number aberrations (CNAs) and differentially expressed genes (DEGs) between both subgroups using aCGH and RNAseq data, followed by molecular characterization through genomic and transcriptomic data integration and gene set enrichment analyses.
Results A total of twelve distinct CNAs were identified between sporadic and radiogenic AS. Chromosome 8q aberrations were of particular interest, as c-MYC alterations have previously been reported in secondary AS.

Five genes—c-MYC, SMURF2, CASC11, PDLIM1, and ZNF280B—were consistently deregulated at both the genome and transcript levels. Additionally, SMURF2 CNA and overexpression were related to poor prognosis and increased metastasis rates. Co-enrichment of E2F and MYC target gene sets, alongside elevated M phase, DNA repair, and mitotic progression pathways, suggests a highly proliferative tumor phenotype of radiogenic AS, characterized by accelerated cell cycle progression, metabolic reprogramming, replication stress, and genomic instability.
Conclusions This multi-level molecular study revealed distinct genomic and transcriptomic patterns in radiogenic AS, which are associated with a more aggressive tumor phenotype compared to sporadic primary AS. The key findings pointed to potential markers for diagnosis and to subgroup-specific therapeutical targets for personalized treatment of AS.
Trial registration and/or ethics approval number Ethical approval (EA) for this study was obtained by the ethics committee of the LMU (18-830).

Young investigators' session: Replace, reduce, refine—Innovative strategies for translational radiation research

E-O1

Modelling resistance to therapy using an oral cancer organoid biobank

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Radiation Oncology 2025, **20(s1)**:E-O1

Background Oral squamous cell carcinoma (OSCC) is the most common subtype of head and neck cancer and arises from different anatomical sites in the oral cavity. These sites exhibit heterogeneous morphologies, and the prevalence of OSCC varies significantly between anatomical locations; however, the underlying mechanisms driving this oral site specificity remain largely unexplored, hindering the development of improved treatment options. Apart from surgery, current therapies—such as chemotherapy, radiotherapy, and immunotherapy—have not shown significant improvements in overall patient survival, which remains below 50%. Elucidating the molecular mechanisms that lead to oral site-specificity and inter- and intra-patient heterogeneity in OSCC is crucial for developing more effective treatment strategies and enhancing patient outcomes.
Materials and methods We have established a biobank of patient-derived organoids (PDOs), which are derived from tissue samples obtained from OSCC patients [1,2]. These samples include normal oral mucosa, as well as primary, recurrent and metastatic OSCC tissue. We characterised the PDOs using histology, immunofluorescence staining, real-time PCR, and RNA sequencing [2]. PDO lines were then used in drug and radiochemotherapy screens to identify OSCC organoids that were sensitive or resistant to therapy [1].
Results OSCC-derived PDOs from different oral sites can be cultured long-term and recapitulate the histology of the tumour epithelium of origin, as well as robustly express biomarkers such as CDH1, KRT14, P63, and MKI67. In drug and radiochemotherapy screens, we have identified PDO-specific responses to chemotherapeutic agents that reflect tumour heterogeneity between patients. We are currently using our patient-matched PDOs to explore the intra-patient drug responses of primary, relapsed and metastatic OSCC, as well as the underlying molecular mechanisms.
Conclusions Our living biobank comprises a diverse array of patient-derived OSCC samples, providing a powerful resource to enhance treatment strategies and improve patient survival rates by facilitating the development of more effective screening methods. Using this approach, we aim to bridge the gap between in vitro findings and clinical applications, ultimately providing patients with more personalised and targeted treatment options.
Trial registration and/or ethics approval number The patient samples were collected at the University Hospital Würzburg dental clinic following written informed consent. Ethical approval was granted by the Ethical Board of the University of Würzburg (protocol number: 218/16, amendment 2021).
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E-O2

The use of the CAM model to access tumor heterogeneity

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Background The chorioallantoic membrane (CAM) assay enables the transplantation of tumor cells onto the highly vascularized membrane of the chicken embryo, which serves as a natural bioreactor enriched with growth-promoting factors. Tumors that form on the CAM can be harvested as either fresh or formalin-fixed tissue for downstream analyses. This model is particularly well-suited for studying tumor growth dynamics, invasive behavior, and metastatic potential. Notably, the CAM assay offers a unique opportunity to spatially resolve intratumoral heterogeneity (ITH)—for example, by comparing the tumor center with the invasive front. ITH arises from clonal evolution during tumor progression and is a key driver of therapy resistance in colorectal cancer (CRC), often accompanied by high cellular plasticity, especially in the context of invasion.
Materials and methods I will present the standard operating procedures (SOPs) and experimental setup of the CAM assay, including a video demonstration of key steps in tumor transplantation. Several applications will be highlighted, including ITH analysis of the methyltransferase EZH2 and modulation of tumor growth under metabolic stress. In addition, I will introduce a spatial transcriptomics approach (NanoString) to identify novel cancer- associated genes in collectively migrating tumor cell clusters at the invasive front.
Results Our findings demonstrate that the CAM assay can be effectively used to generate hypotheses, validate in vitro observations, and confirm biological relevance in a dynamic, in vivo-like environment.
Conclusions Since chicken embryos are not classified as animals under current legislation prior to hatching, the CAM assay aligns with the 3Rs principle (Replace, Reduce, Refine). It therefore represents an ethically responsible and highly informative alternative to conventional animal models in cancer research.

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E-O3

Advancing preclinical radiotherapy research using 3D bioprinted lung cancer models and experimental irradiation platforms

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Background Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) constituting the majority of cases. Radiotherapy plays a crucial role in treatment; however, preclinical evaluation of novel radiation techniques is hampered by the limitations of conventional 2D models and ethical concerns associated with animal testing. The emergence of three-dimensional (3D) bioprinting offers an innovative approach to replicate tumor microenvironments and enhance the translational relevance of radiobiological studies.

Materials and methods Three studies were integrated to evaluate and enhance 3D bioprinted lung cancer models for radiotherapy research. First, we established 3D bioprinted constructs of A549 NSCLC cells using a gelatin-alginate-Matrigel matrix, validated for mechanical stability and radiobiological responsiveness under high-dose microbeam and broad-beam irradiation at synchrotron beamlines [1]. Second, to simulate in vivo conditions, bioprinted tumors were embedded in a 3D-printed mouse phantom mimicking murine tissue density and radiological properties, and irradiated under clinical X-ray beam setups [2]. Third, we developed perfusable, vascularized 3D organ models via a novel sacrificial-free direct ink writing (SF-DIW) technique, which enabled precise fabrication of hollow vascular channels without compromising structural integrity or cell viability. These models were integrated with a custom perfusion system to sustain long-term culture and dynamic drug testing [3].

Results In the first study, 3D constructs withstood synchrotron-based irradiation and allowed spatial discrimination of DNA damage (γH2AX foci), apoptosis, and metabolic activity across irradiated and non-irradiated regions. Microbeam irradiation induced localized cell death confined to peak dose zones, a distinction unresolvable in 2D cultures. Models embedded in a mouse phantom for the second study demonstrated dose distributions comparable to in vivo settings, with increased accuracy in simulating thoracic radiotherapy. Finally, the SF-DIW approach yielded perfusable constructs that supported tumor-stroma interactions and drug metabolism studies. Perfused models exhibited superior viability, physiological relevance, and drug response compared to static cultures.

Conclusions The integration of 3D bioprinting with synchrotron-based microbeam irradiation, radiologically accurate phantoms, and perfusion systems provides a robust and ethically viable platform for preclinical radiotherapy research. These advanced in vitro models recapitulate tumor microenvironments more effectively than 2D cultures or static 3D models, and hold promise for evaluating novel therapeutic strategies, including high-dose rate and spatially fractionated radiotherapy techniques.

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