or p16 negative cells, indicating a differential role of p16 protein expression depending on its localization. Strikingly, cells expressing nuclear p16 (p16-NLS) -although showing a similar level of gH2AX induction- were characterized with lower number RAD51 foci formation compared to cells expressing cytoplasmic p16 (p16-NES), suggesting an impaired HRR.

Conclusion: Cellular p16 localization is an important factor for stratification of HNSCC patients with nuclear p16 expression showing a superior predictive value for radiotherapy response.

OC-0440

Impact of chemokine receptor CXCR4 and its ligand SDF1 expression on loco-regional control in HNSCC

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Purpose or Objective: To retrospectively assess the prognostic value of the potential biomarkers, i.e. chemokine receptor CXCR4, its ligand CXCL12 (SDF1), and nuclear EGFR expression in a cohort of 201 patients with locally advanced HNSCC. Patients were treated between 2005 and 2011 in 8 German cancer centers, as part of a multicenter biomarker study of the German Cancer Consortium Radiation Oncology Group (DKTK-ROG). Experimental data and first clinical observations suggest that activation of CXCR4 and SDF1 signaling pathway and nuclear location of EGFR are implicated in tumour cell proliferation, cellular survival, tumour progression, worse overall survival, metastasis and enhanced treatment resistance in different tumour types.

Material and Methods: Patients with locally advanced SCC of the oral cavity, oropharynx and hypopharynx were treated with resection and adjuvant RT and Cisplatin-based CT. Tissue micro-arrays (TMAs) were generated from surgical specimens and evaluated for the expression of the biomarkers by immunofluorescence with a semi-quantative method, based on their cellular location (membranous, intracellular, nuclear), extent of expression on TMA area and staining intensity. The results of the biomarker analysis along with the clinical parameters were then correlated with the clinical outcome.

Results: In univariate analysis, tumours with either SDF1 or CXCR4 intracellular overexpression displayed a significant negative correlation with loco-regional control (LCR) (HR: 2.52, p=0.01 and HR: 1.96, p=0.05 respectively). No correlation was observed for the nuclear expression of EGFR (HR: 0.85, p= 0.67), membranous expression of SDF1 (p=0.73) or CXCR4 (p=0.38). Tumours with intracellular co-expression of both SDF1 and CXCR4 were significantly correlated with poor LRC (HR: 2.72, p=0.01). Previously published data from the same cohort, showed that absence of p16 (negative HPV status) was correlating with poor LRC. Importantly, increased expression of SDF1 or co-expression with CXCR4 could identify a group of patients with significantly worse outcome within the HPV negative group (p=0.01). Multivariate cox regression analysis including HPV status, tumour localisation, tumour volume and the respective biomarkers indicated a significant independent role of SDF1 (HR: 2.20, p=0.04) and co-expression with CXCR4 (HR: 2.19, p=0.05) on LRC.

Conclusion: In summary, pre-treatment overexpression of CXCR4/SDF1 is an independent negative prognostic factor for the outcome of patients with locally advanced HNSCC who receive surgery and standard RT-CT. Further investigation in a cohort of patient receiving primary RT-CT and a prospective validation study is currently ongoing. SDF1/CXCR4 appears to be a promising biomarker for treatment individualization, in particular in HPV negative advanced HNSCC patients and supports strategies using drugable targets against this pathway to enhance efficacy of standard treatment.

OC-0441

Genomic amplification of FancA in HNSCC: mechanisms of radioresistance and clinical relevance

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Purpose or Objective: Radio(chemo)therapy is a crucial treatment modality for head and neck squamous cell carcinoma (HNSCC). Radiotherapy resistance is a major reason for therapy failure and, therefore, predictive biomarkers for therapy response are urgently needed. DNA gains on chromosome 16q23-24 have been shown to be associated with genomic amplification of the FancA gene and to correlate with reduced progression-free survival of HNSCC patients after radiotherapy. This study aimed to analyze the effects of the potential predictive marker FancA on radiation sensitivity *in vitro*, to characterize the underlying molecular mechanisms, and to evaluate the clinical relevance in HNSCC.

Material and Methods: We generated FancA overexpressing human oral keratinocytes (OKF6/FancA) and analyzed several endpoints upon irradiation. To identify signaling pathways involved in FancA-mediated resistance, global transcriptome analyses were performed after irradiation with 4 Gy or sham-irradiation followed by pathway enrichment analysis and reconstruction of function interaction networks. The clinical relevance of the cytogenetic marker 16q23-24, the FancA gene and our *in vitro* results was analyzed in data of 113 radiotherapy-treated patients from The Cancer Genome Atlas (TCGA) HNSCC cohort (Nature, 2015).

Results: Overexpression of FancA resulted in enhanced survival after *in vitro* irradiation. Moreover, FancA overexpressing cells demonstrated accelerated DNA damage repair mechanisms paralleled by increased repair fidelity: enhanced p53 and p21 response, accelerated kinetics in the disappearance of γ-H2AX DNA damage repair foci, faster pATM translocation, reduced accumulation of chromosomal translocations, but no increase in FancD2 mono-ubiquitinylation. Global mRNA expression analyses identified interferon signaling as a major candidate pathway, which was affected by FancA overexpression. Functional interaction networks of genes deregulated upon irradiation pointed to pathways exclusively involved in FancA-mediated radioresistance including the *senescence-associated secretory phenotype (SASP)*. Increased levels of basal and irradiation-induced cellular senescence accompanied by enforced SASP formation further support their potential involvement in FancA-mediated radiation resistance. The clinical relevance of our findings was validated in the data of 113 radiotherapy-treated patients of the TCGA HNSCC cohort demonstrating the association of chromosomal gains on 16q24.3 with increased FancA mRNA expression levels and impaired overall survival. Furthermore, the translation of our *in vitro* model derived results into the HNSCC patient specimens revealed similar gene expression changes linked to FancA overexpression.

Conclusion: Our data suggest an important role for FancA in cellular mechanisms of radioresistance in HNSCC.

OC-0442
Does miR-210 predict benefit from hypoxia modification in BCON randomised bladder cancer patients?

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Purpose or Objective: The addition of hypoxia modifiers carbogen and nicotinamide (CON) to radiotherapy (RT) improved overall survival in bladder cancer patients enrolled in the BCON phase III clinical trial. We investigated whether the expression of miR-210 in the BCON patient samples reflects hypoxia and predicts benefit from hypoxia-modification.

Material and Methods: The retrospective study involved 183 T1-T4b patients: 86 received RT+CON and 97 received RT alone. Formalin-fixed samples taken prior to radiotherapy were available and RNA extracted. Customised TaqMan plates were used to assess miR-210 expression using quantitative real-time PCR. Patients were classified as low miR-210 (<median expression) or high miR-210 (≥ median). Data on other hypoxia biomarkers were available for comparison.

Results: Patients with high miR-210 had a trend towards improved five-year OS with RT+CON (53.2%) compared with RT alone (37.8%; HR 1.68, 95% CI 0.95-2.95, P=0.08). No benefit was seen with low miR-210 (HR 1.02, 95% CI 0.58-1.79, P=0.97). High expression of miR-210 was also associated with high HIF-1α protein (P=0.0008), CA9 protein (P=0.004), Glut-1 protein (P=0.02), expression of a 26-gene hypoxia signature (P=0.01), tumour necrosis (P=0.04) and concurrent pTis (P=0.03).

Conclusion: High miR-210 expression may reflect tumour hypoxia and should be investigated further as a potential biomarker to identify bladder cancer patients who would benefit from hypoxia-modifying therapies.

OC-0443
Radiotherapy sensitivity in breast cancer is influenced by the DNA cytosine deaminase APOBEC3B

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Purpose or Objective: The DNA cytosine deaminase APOBEC3 proteins catalyze deamination of cytidines in single-stranded DNA, providing innate protection against retroviral replication. Recent studies have implicated APOBEC3B as a major source of mutation in breast cancer, suggesting a role for these enzymes in tumor initiation and/or progression. APOBEC3B expression levels were earlier found to correlate with poor outcomes for patients with estrogen receptor positive breast cancer, especially after Tamoxifen. Given its role in mutagenesis, we set out to assess whether APOBEC3B associates with radiosensitivity in breast cancer.

Material and Methods: MCF7 breast cancer cells were cultured radioresistant by daily 2 Gy treatments or tamoxifen-resistant by continuous culturing in up to 10 uM 4-OH-tamoxifen. The effect of irradiation on expression of APOBECs was assessed by RNAseq and qPCR in radiosensitive and radioresistant MCF7, and by qPCR in radioresistant MDA-MB231 cells. Furthermore, we studied a retrospective cohort of 535 non-systemically treated breast cancer patients. The predictive power of APOBEC3B was assessed in patients that did or did not receive radiotherapy as part of their primary therapy. Next, we suppressed endogenous APOBEC3B in MCF-