PO-490 EXPRESSION OF CORTACTIN AND FOCAL ADHESION KINASE IN EARLY STAGES OF LARYNGEAL TUMORIGENESIS AND CLINICAL APPLICATION FOR CANCER RISK-STRAITIFICATION

1JM García-Pedrero*, 1MA Villaronga, 1F Heredia-Prado, 1R Granda-Diaz, 1ST Menendez, 2M Quez, 3M Vilaseca, 3E Allonca, 1M Sanchez Cantel, 1IP Rodrigo, 1Hospital Universitario Central de Asturias, Department of Otolaryngology- Instituto de Investigación Sanitaria del Principado de Asturias- IUOPA- Universidad de Oviedo- and CIBERONC, Oviedo, Spain; 2Hospital Santa Creu i Sant Pau, Department of Otolaryngology, Barcelona, Spain; 3Bone Clinic, Department of Otolaryngology, Barcelona, Spain

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Introduction Cortactin (CTTN) and the Focal Adhesion Kinase (FAK) are two major candidate genes to respectively drive 11q13- and 8q24-associated aggressive behaviour in various cancers. Recent evidence uncovered their clinical relevance in early stages of tumorigenesis as promising biomarkers for cancer risk assessment.

Material and methods Using a multicenter validation study CTTN and FAK expression was evaluated by immunohistochemistry in a cohort of 109 patients with laryngeal precancerous lesions, and correlated with clinicopathologic parameters and laryngeal cancer risk. The pathophysiologic role of CTTN and FAK was further investigated using functional studies in cellular models.

Results and discussions Increased CTTN and FAK expression was detected in 49 (41%) and 35 (32%) laryngeal dysplasias, respectively. Univariate Cox analysis showed that CTTN and FAK expression but not histological grading were significantly associated with both recurrence risk and laryngeal cancer risk. Patients carrying strong CTTN- or FAK-expressing lesions experienced the highest laryngeal cancer incidence (log-rank p<0.001). In multivariate stepwise analysis, FAK expression (HR=13.91, 95% CI 4.82–40.15; p<0.001) and alcohol consumption (HR=2.22, 95% CI 1.17–4.20; p=0.014) were significant independent predictors of laryngeal cancer development. Targeting FAK by either RNAi or pharmacological inhibitors effectively blocked cell growth, colony formation and invasion into 3D collagen matrices.

Conclusion CTTN and FAK emerge as powerful predictors of laryngeal cancer risk beyond histological grading, thus encouraging their clinical application as complementary markers for risk-stratification. Furthermore, our findings unveil that pharmacological targeting of FAK could constitute a promising therapeutic strategy for HNSCC prevention and treatment.

PO-491 FREQUENT MTORC1 ACTIVATION IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS CORRELATES WITH FAVOURABLE CLINICAL OUTCOME

1F Heredia-Prado, 1D Garcia-Carracedo, 1MA Villaronga, 1S Alvarez-Teijero, 1S Santamaria, 4MV Gonzalez, 1MB Balbin, 1A Astudillo, 1P Rodrigo, JM Garcia-Pedrero. Hospital Universitario Central de Asturias, Department of Otolaryngology- Instituto de Investigación Sanitaria del Principado de Asturias- IUOPA- Universidad de Oviedo- and CIBERONC, Oviedo, Spain; 2Columbia University Medical Center, Herbert Irving Comprehensive Cancer Center, New York, USA; 3Hospital Universitario Central de Asturias, Department of Molecular Oncology- Instituto Universitario de Oncología del Principado de Asturias- Universidad de Oviedo, Oviedo, Spain; 4Hospital Universitario Central de Asturias, Department of Pathology- Instituto Universitario de Oncología del Principado de Asturias- Universidad de Oviedo, Oviedo, Spain

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Introduction The PI3K/AKT/mTOR signalling pathway has emerged as one of the most frequently deregulated in head and neck squamous cell carcinomas (HNSCC), making it very attractive for molecular-targeted therapies. Numerous alterations of various upstream and downstream components have been described; however, their prognostic significance and impact on HNSCC patient survival remains to be established.

Material and methods We performed a comprehensive study to investigate the prognostic significance of multiple genetic and biochemical alterations in key players of the PI3K/AKT/mTOR pathway (i.e. PI3CA and AKT1 mutations and immunohistochemical expression of EGFR, PDK1, p-AKT, PTEN and p-S6) using an unbiased cohort of 93 consecutive and homogeneous surgically treated HNSCC patients. Results were validated in an independent cohort of 432 HNSCC patients.

Results and discussions Our findings reveal the high prevalence of S6 phosphorylation, a surrogate marker of mTORC1 activation, in HNSCC specimens (>70%) and, more importantly, demonstrate its relevance on clinical outcome. Phosphorylation of ribosomal protein S6 on either Ser235/236 or Ser240/244 was consistently and significantly correlated with favourable prognosis, although with differences depending on the tumour site. Thus, p-S6 expression was significantly correlated with better disease-specific survival specifically in the subgroup of laryngeal carcinoma patients (p<0.001). In addition, multivariate regression models revealed p-S6 to be an inverse and independent predictor of lymph-node metastasis (p=0.004) and distant metastasis (p=0.006).

Conclusion Taken together, this study unveils an unprecedented correlation of mTOR activation with improved clinical outcome in patients with laryngeal carcinomas and uncovers the potential of p-S6 expression as a good prognostic biomarker and an inverse predictor of lymph node and distant metastases. These results should be of broad interest as immunohistochemical detection of p-S6 may help to stratify patients and guide treatment decisions.

PO-492 EX VIVO FUNCTIONAL HOMOLOGOUS RECOMBINATION (HR) TEST DETECTS BRCA REVERSAL AND RESISTANCE TO PARPI IN METASTATIC BREAST CANCER PATIENTS

1T Meijer*, 1N Verhaak, 2C Van Deurzen, 3H Dubbink, 2D Den Toom, 2W Dinjens, 1R Karasar, 2D Van Gent, 1A Jager. Erasmus Medical Center, Molecular Genetics, Rotterdam, The Netherlands; 2Erasmus Medical Center, Pathology, Rotterdam, The Netherlands; 3Erasmus Medical Center, Medical Oncology, Rotterdam, The Netherlands

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Introduction Better predictive biomarkers for response to Poly ADP-Ribose inhibitors (PARPi) are required, since on the one hand evidence is emerging that PARPi are also effective beyond germline BRCA mutated (gBRCAm) cancers and on the other hand gBRCAm cancers can become resistant to PARPi. Therefore, we previously developed a functional homologous recombination (HR) assay exploiting the formation of RAD51 foci in proliferating cells after ex vivo irradiation of fresh primary breast cancer (BrC) tissue (n=148): the REpair CAPacity (RECAP) test. The aim of the current study is to validate feasibility of this test on histological biopsies from metastatic BrC and to explore the utility of the RECAP test as a predictive biomarker for PARPi treatment of metastatic BrCs.

Material and methods Fresh tissue biopsies from metastatic BrC lesions were collected in customised DMEM/F12 medium, irradiated with 5 Gy and cultured for 2 hours. Molecular
characterisation of functional HR deficient (HRD) biopsies as well as platinum/PARP resistant biopsies was performed.

**Results and discussions** 41 biopsies were derived from 38 patients with recurrent or metastatic BrC. The RECAP test had a high success rate (93%) when performed on core needle or punch biopsies. Final test outcomes were available within 1 week after the biopsy procedure. HRD was detected in 13 out of 41 biopsies (32%). Among these 13 HRD tumours, 5 were gBRCAm, indicating that the RECAP test identifies more patients who may benefit from PARPi treatment than germline BRCA analysis only. In three gBRCAm patients BRCA reversion was detected, as the HRD tumours became HR proficient (HRP) after showing in vivo progressive disease (PD) on cisplatin/PARPi treatment. One of these patients obtained a secondary BRCA1 mutation that restored the open reading frame and led to production of full-length BRCA1 protein, while the causative molecular event in the other patients is still elusive.

**Conclusion** The RECAP test is a functional HRD test that reflects the real-time HR status regardless of BRCA mutational status. Thus, RECAP shows great potential as a predictive biomarker for PARPi treatment of metastatic BrC.

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**PO-493** CTDNA PROFILES OF METASTATIC MELANOMA PATIENTS UNDER THERAPY

1 R Varaljai*, 2 N Von Neuhoff, 3 A Sucker, 4 J Newton-Bishop, 5 D Schadendorf, 6 A Roesch.

1 University Hospital Essen, Department of Dermatology, Essen, Germany; 2 University Hospital Essen, Department of Pediatric Hematology-Oncology, Essen, Germany; 3 University of Leeds, Leeds Institute of Cancer and Pathology, Leeds, UK

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**Introduction** In precision oncology it is a great interest to develop novel disease and therapy monitoring technologies that are non-invasive and highly sensitive. Specifically, our project aims to establish blood-based assays that allow ‘real-time’ monitoring and quantitative detection of circulating tumour DNA (ctDNA) as a biomarker that corresponds to the tumour load in patients. ctDNA fragments are released from all parts of the tumours by apoptosis and necrosis, thus, it reflects the full spectrum of specific mutations of a systemically progressed tumour.

**Material and methods** The usefulness of ctDNA analyses is highlighted in this study, in which we have analysed 545 plasma samples from 77 stage III and IV melanoma patients with the BRAFV600E mutation, with mutations at the Q61 codon of the NRAS gene, and mutations in the promoter region of TERT gene. Characterisation and analysis of these mutations were performed on droplet digital PCR (ddPCR) platform. The patients received either MAPK-targeted treatment or immune checkpoint blockade. Additionally, plasma samples from 96 healthy donors were analysed to test the positive and negative predictive values of our assays.

**Results and discussions** ROC analyses showed over 90% AUC for all our assays. Our analyses revealed that increasing ctDNA levels were associated with disease progression from loco-regional (IIIB or IIIC) to systemic disease (IV) with p<0.05. We evaluated our ctDNA assays with bio-statistical methods, where ctDNA levels were correlated with treatment response and progression free survival. ctDNA levels during therapy corresponded to the radiologic tumour load from CT and MRI scans. Moreover, ctDNA levels often indicated disease progression earlier than the routine radiological scans.

**Conclusion** In brief, our results show the potential role of ctDNA measurement as a sensitive monitoring tool for the early assessment of disease progression and therapeutic response/resistance in melanoma patients.

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**PO-494** A PATHOGENIC NETWORK OF NOVEL MOLECULAR MECHANISMS DRIVING MERKEL CELL CARCINOMAS. SHARED ONCOGENIC PATHWAYS BETWEEN VIRUS-POSITIVE AND UV-INDUCED TUMOURS

1 C Gonzalez-Vela, 2 S Curiel-Olmo, 3 MA Piñis, 4 JP Vaqué*, 5 idival, pathology, Santander, Spain; 6 idival, cancer genomics, Santander, Spain; 7 idival, pathology, santander, Spain; 8 University of Cantabria, Molecular biology, Santander, Spain

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**Introduction** Merkel cell carcinoma (MCC hereon) is a highly malignant neuroendocrine skin tumour. MCC has a relatively low incidence, but its mortality exceeds that of melanoma. MCC shares important features with other deadly cancers like SCLC or melanoma. Recent data have shown important mechanisms driving MCC. Nevertheless, the main molecular/biological mechanisms involved in its pathogenesis and clinical course remain essentially unveiled. In this work, we aimed at studying a number of MCC clinically characterised cases to identify potential new biomarkers for diagnosis and therapy.

**Material and methods**
- NGS-Sequencing (WES) from a cohort of 15 clinically characterised patients.
- Bioinformatic analysis to detect altered genes and signalling pathways.
- Immunohistochemistry assays in a cohort of 50 clinically characterised patients.
- Multivariable survival study.
- Functional analyses in MCC cell lines.

**Results and discussions** We detected two groups with differential genetic characteristics: 1) MCC-MCPyV+; Expressing MCPyV and with a low mutational load (0.2–1 muts/Mb) and 2) MCC-MCPyV-; Without the virus but with a high mutational load (10–25 muts/Mb, similar to that of melanoma or lung cancer). Moreover MCC-MCPyV- tumours harboured typical U.V. mutational signatures with up to 60% C>T transitions in a dyspirimidine context as recently published by others. Studying a number of transcription factors as end-points for the activity of the signalling pathways associated to the mutated genes found in our study, we found, that despite the differences between MCPyV + and MCPyV- tumours, both developed similar mechanisms of disease like P-CREB and P-STAT. Also MCPyV-cases (recently associated with more aggressiveMCCs) also developed alternative almost exclusive mechanisms like YAP, MYC and LEMI. We propose that these signalling proteins can serve as biomarkers for diagnosis (prognosis) and therapy. As part of our results a multivariable analysis identified P-CREB as independent survival biomarker. Also we have detected multiple mechanisms that can trigger P-CREB in MCC cells. Intriguingly, an algorithm that combined MCPyV-negative mechanism also showed poor survival outcome within our cohort of patients.

**Conclusion** In the era of targeted and immune therapies, we need specific biomarkers. Despite important genetic differences between MCPyV +and MCPyV- tumours we show that both share important mechanisms of disease. Amongst these, P-CREB was an independent survival factor and can serve for diagnosis and therapy of MCC and related tumours.