but, on the long run, leads to the convergent evolution of aggressive clones with mitotic checkpoint defects. In line with this, downregulation of PI3K-C2α promotes spindle alterations and aneuploidy, indicating that PI3K-C2α expression is a key determinant of genomic stability. As a consequence of the altered spindle, reduction of PI3K-C2α expression increases the sensitivity to anti-MT drugs, such as paclitaxel, in pre-clinical models and in breast cancer patients.

**Conclusion** Loss of PI3K-C2α expression is a frequent occurrence in breast cancer patients (48%) and correlates with local recurrence and metastatic disease. The heterogeneous loss of PI3K-C2α initially delays tumour onset but, on the long run, leads to the convergent evolution of aggressive clones with mitotic checkpoint defects. In line with this, downregulation of PI3K-C2α promotes spindle alterations and aneuploidy, indicating that PI3K-C2α expression is a key determinant of genomic stability. As a consequence of the altered spindle, reduction of PI3K-C2α expression increases the sensitivity to anti-MT drugs, such as paclitaxel, in pre-clinical models and in breast cancer patients.

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### Abstracts

**PO-467** UNCLARIFIED CASES OF MICROSATellite INSTABILITY ANALYSES FOR LYNCH SYNDrome DIAGNOSTICS

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**Introduction** Lynch syndrome (LS) is an autosomal dominantly inherited form of colorectal cancer (CRC) and is implicated in 2%–4% of CRC cases. LS develops from a mutation in one allele of one of the DNA mismatch repair (MMR) genes, most commonly MLH1 and MSH2, or less frequently MSH6 and PMS2. Loss of functional MMR proteins leads to defects in DNA repair and, subsequently, high DNA microsatellite instability (MSI-High). LS diagnostics currently consists of an analysis of MMR protein expression by means of Immunohistochemistry (IHC) and a molecular analysis to detect MSI. During our 10 year experience in performing LS diagnostics we have encountered few cases where IHC and MSI analyses do not match. This study is to clarify these inconsistent molecular alterations which will contribute to our understanding of LS diagnostics.

**Material and methods** Of 2335 LS cases 27 (1.1%) showed discrepant IHC and MSI results. To clarify this, IHC and MSI analyses were repeated if possible with different antibodies and different MSI markers (mononucleotide instead of dinucleotide markers). Using MS-MLPA MLH1 hypermethylation, BRAF V600E mutation status and LOH of the MMR genes was analysed. Finally, germline and somatic mutations in MMR genes were analysed by using NGS. Protein modelling was also performed to visualise structural protein changes.

**Results and discussions** Among these 27 cases, due to the decline of patients for further testing, 4 cases were excluded from further analyses. Of the remaining cases in 2 cases using a different antibody explained the unexpected results and in 2 cases switching from dinucleotide to mononucleotide MSI markers. In respectively 8 and 4 cases somatic and germline mutations explained the IHC and MSI results. Finally, in 2 cases protein modelling of identified mutations explained the presence of protein staining in MSI-high cases. The rest cases cannot be further studied due to the lack of tissues.

**Conclusion** Based on these results, we have concluded: 1. Mononucleotide markers are more sensitive to detect microsatellite instability. 2. Protein modelling can explain presence of protein staining for mutations that do not cause a major conformational change. 3. Somatic sequencing is a valuable addition to Lynch syndrome diagnostics.

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**PO-468** LAMININ332 (α3; β3; γ2) GENES AND PROTEIN EXPRESSION IN CERVICAL CARCINOMAS

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**Introduction** Laminin332 (α3; β3; γ2 chains) has been identified as an important macromolecule in cancer invasion and metastasis, influencing cell differentiation, migration, adhesion, cell proliferation and survival.

The α3, β3, and γ2 chains of LAM-332 are encoded by three distinct genes, LAMA3, LAMB3, and LAMC2, respectively. LAMC2 is associated to the invasive and metastatic abilities of several tumour types such as colon and pancreas. However, the molecular role of LAMC2 in cervical carcinomas has not yet been fully elucidated.

We investigated the functional significance of LAMC2 in cervical carcinomas: squamous cell carcinomas (SCC) and adenoscarcinoma (ADC) as well as its role in SCC of the cervix.

**Material and methods** SiHa (SCC) and HeLa (ADC) cell lines were used. The expression of LAMA3, LAMB3, LAMC2 and EGFR was evaluated by qPCR in the presence/absence of EGF. Lamα2 levels were evaluated by immunofluorescence. Lamα2 role in migration and invasion was evaluated by wound healing and trans-well assays in control, EGF stimulated and LAMC2 silenced cells (shRNA). Immunohistochemical study evaluated lamα3, β3, and γ2 chains and EGFR, in formalin-fixed, paraffin-embedded in 122 cases, both in situ (n=15) and invasive cervical carcinomas: SCC (n=106) and ADC (n=41). Fisher’s exact test was performed for statistical analysis.

**Results and discussions** EGF stimulates the migration and invasion in SiHa cells. However knocking-down Lamα2 decreases cell migration and invasion capacities in SiHa cells. Also, the knockdown of LAMC2 lead to an increased transcription of LAMA3 and LAMB3 genes only in SiHa. The immunohistochemical study demonstrated that lamα2 chain in SCC cases was significantly associated with invasion (p=0.0001) and with that histological type (p=0.0183). In SCC cases, there was also a significant association of lamα3 and γ2 chains overexpression (p=0.008). Lamα3 was associate with ADC type (p<0.0001). EGFR immuno-overexpression was also associated with SCC (p<0.0001).

**Conclusion** Laminin332 and EGFR are differently expressed in the two most common histological types of cervical carcinomas (SCC and ADC). Lamα2 expression is associated with SCC in both in vitro and clinical context and Lamγ2 -
dependent invasion is probably mediated by EGFR dependent signalling pathways.

Together, results demonstrate a role of extracellular matrix proteins in the progression of cervix carcinoma. Additionally, Siha and HeLa can be an effective in-vitro models for an overview of the processes involved in cervical cancer progression.

### Poster Presentation: Translational Research Biomarkers

#### PO-471 CIRCULAR RNA DETECTION IN MELANOMA PATIENTS’ PLASMA

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Introduction Melanoma is an aggressive disease, curable by surgical resection if caught at an early stage (>95% 5 year survival) but with a poor prognostic outcome when diagnosed at advanced metastatic stage (<5% 5 year survival). Therefore, there is an urgent need for biomarkers of early diagnosis. In this respect, liquid biopsies hold great promise as they are non-invasive and allow repeated sampling and monitoring of patient response.

Circular RNAs (circRNAs) are covalently closed RNA molecules that are more stable than linear RNAs to RNase activity in blood, and therefore have great potential as circulating biomarkers for cancer patient management. We therefore set out to identify and validate potential biomarker circRNAs from the plasma of melanoma patients representing differing stages of the disease, along with healthy controls.

Material and methods Next generation sequencing (RNAseq) was performed on pools of plasma from melanoma patients with stages 0, I/II, III or IV disease, as well as healthy individuals. Back-spliced junction reads were mapped using a combination of TopHat and TopHat-Fusion, and differential expression analysis carried out with the DEseq algorithm. Three circRNA (CR-3320, CR-2465 and CR-4452) were selected – 20 circRNA species were identified between 120 melanoma patients by NanoString nCounter platform and validated with digital droplet (dd)PCR in serum, plasma and exosomes. ISH confirmed dysregulation in 28 miRs was associated with PFS and OS. Among these miRs, up-regulation of miR-652 might be exploited as biomarkers for the upfront selection of patients’ candidate to regorafenib treatment and might be used to track and forecast acquired resistance to treatment.

Conclusion This study suggests that circRNAs could represent a novel source of biomarkers for liquid biopsies for melanoma, and in all probability other cancers.

#### Poster Presentation: Translational Research Biomarkers

#### PO-472 MICRORNA AS BIOMARKERS OF RESISTANCE TO REGORAFENIB IN METASTATIC COLORECTAL CANCER PATIENT

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Introduction Regorafenib demonstrated efficacy in pre-treated metastatic colorectal cancer (mCRC) patients. Limited clinical benefit in unselected patient populations highlights the unmet need for better patient selection and identification of mechanisms of action. MicroRNAs (miRs) are small non-coding RNAs involved in cell homeostasis, carcinogenesis and control multiple oncogenic pathways. Numerous miRs deregulation in mCRC are associated with clinical outcome and cancer progression.

Material and methods We ran a translational phase II trial of regorafenib in chemo-refractory mCRC patients with biopsiable metastases. Tissue biopsies were obtained at baseline (BL), after 2 months of treatment, and at disease progression (PD). Patient Derived Organoids (PDOs) and PDO-xenotransplants were generated to study primary and acquired resistance to regorafenib. MiR profiling was performed in BL serum of all patients by NanoString nCounter platform and validated with digital droplet (dd)PCR in serum, plasma and exosomes and by In Situ Hybridization (ISH) in matching tissue biopsies. Fisher’s exact test investigated potential associations between patient groups and categorical variables whilst t-test or non-parametric equivalent tests were used for continuous variables. Progression Free Survival (PFS) was measured from date of registration to date of first progression/relapse or death from cancer progression. Overall Survival (OS) was measured from date of randomisation to death from cancer. The Kaplan-Meier method summarised the survival estimates while the Cox proportional hazards model used to compare the survival rates between patient groups with and without adjustment for the effect of covariates.

Results and discussions MiR expression was tested in 43 BL sera. Dysregulation in 28 miRs was associated with PFS and/or OS. Among these miRs, up-regulation of miR-652–3 p and down-regulation of miR-3614–3 p was associated with worse PFS and OS. Results were validated by ddPCR on the same serum samples, and matching plasma. ISH confirmed dysregulation of two miRs in sequential tissue biopsies, PDOs and PDO-xenotransplants of patients with primary and acquired resistance. Validation in an independent patients’ cohort (n=70) is ongoing. Functional experiments to define miR-mediated resistance are ongoing.

Conclusion Circulating miR-652–3 p and miR-3614–3 p might be exploited as biomarkers for the upfront selection of patients’ candidate to regorafenib treatment and might be used to track and forecast acquired resistance to treatment.