Results and discussions L-resistant clones were obtained. Resistance was confirmed by MTT analysis. Data obtained by microarray were analysed by principal component analysis to determine the significant sources of variability in the data sets. Gene expression changes are clearly observed between two resistant clones versus parental line. Impressively, 132 genes were significant differentially expressed among resistant clones versus parental line. Unsupervised hierarchical clustering of 132 genes revealed a robust classification between three different groups. When analysed in details, it was possible to identify a large number of genes regulated by NFR2, a master transcriptional regulator that activates genes involved in oxidative stress response, detoxification, and drug resistance. NFR2 expression was evaluated by Western Blot analysis among both L and T resistant cells. After subcellular fractionation, it was possible to observe a nuclear overexpression among resistant cell lines, this data was confirmed by IF. When siRNA of NFR2 was performed, a decrease of cell growth among resistant cell lines was observed. When treatment with L was administered in knockdown cells, it was possible to restore sensitivity.

Conclusion NFR2 is identified as a new mechanism of resistance to antiHER2 inhibition in GC. The evaluation of its expression among xenograft models and patients, who experienced a disease progression, are ongoing.

Poster Presentation: Experimental/Molecular Therapeutics, Pharmacogenomics

PO-501 DECONSTRUCTING THE ROLE OF CD44 IN GASTRIC CANCER RESISTANCE TO CISPLATIN

1,2C Pereira*, 1,3,4D Ferreira, 3,4P Granja, 1,2,5G Almeida, 1,2,5C Oliveira. 1Institute of Molecular Pathology and Immunology, Expression Regulation in Cancer, Porto, Portugal; 2Institute for Research and Innovation in Health Sciences, Biomaterials for Multistage Drug and Cell Delivery, Porto, Portugal; 3National Institute of Biomedical Engineering, Biomaterials for Multistage Drug and Cell Delivery, Porto, Portugal; 4Institute for Research and Innovation in Health Sciences, Biomaterials for Multistage Drug and Cell Delivery, Porto, Portugal; 5Faculty of Medicine- University of Porto, Department of Pathology and Oncology, Porto, Portugal

Introduction Gastric cancer (GC) is the 3rd leading cause of cancer related deaths and the 5th commonest cancer worldwide, affecting ~1 million individuals per year. For early-stage disease, surgical resection is potentially curative, however >80% of GC patients present advanced, unresectable and not curable disease, and an average overall survival (OS) of ~1 year. Poor patient survival is justified by late stage diagnosis and by poor response to therapy. We hypothesised that some GC clones that intrinsically resist to chemotherapy overexpress a variant of CD44, the main cell surface receptor for hyaluronic acid. Supporting this hypothesis is our finding that CD44v6 becomes overexpressed in ~70% of all GCs, as opposed to normal mucosa. Here, we aim to investigate whether CD44v6 overexpression influences response to cisplatin treatment.

Material and methods We established isogenic GC cell lines overexpressing either CD44v6, CD44std or an empty vector (Mock), and CD44v6 RNAi-depleted GC cell lines that endogenously express CD44v6. These were all characterised by RT-PCR, western-blot, immunofluorescence and flow-cytometry. The effect of cisplatin on cell survival was evaluated by SRB and Annexin-V assays. The expression of signalling partners downstream of CD44 was evaluated by western-blot and immunofluorescence.

Results and discussions CD44v6 overexpression increased cisplatin resistance and its depletion sensitised cells to cisplatin treatment. Moreover, when isogenic CD44v6-expressing cells were co-cultured with the non-expressing counterpart, treated with cisplatin and allowed to recover for 15 days, CD44v6-expressing cells survived, while CD44v6-negative cells were reduced to <20% of the cells in culture. We then investigated pathways downstream of CD44v6 that could be involved in cisplatin resistance. We found that CD44v6 overexpression promotes early activation of phospho-Stat3 in one model, which likely confers a survival advantage, and in another model, we found that phospho-p38 locates at the nucleus if CD44v6 is overexpressed, a mechanism commonly associated with cisplatin resistance.

Conclusion In conclusion, our findings highlight CD44v6 as a modulator of cisplatin resistance in GC that may contribute to the poor therapeutic response in this disease. Novel therapeutic strategies that include CD44v6 depletion at the tumour site, may improve therapy response and survival in GC patients.

PO-503 HDAC INHIBITOR RESISTANCE IN COLORECTAL CANCER: RAS AND AMP; MYC – THE PARTNERS IN CRIME

1Ispanianie*, 2S Kistler, 3A Heberle, 4F Uhlig, 5K Kasack, 6G Dittmar, 7N Blüthgen, 1K Thiediek, 2S Campbell, 3L. Sers. 1Charité-Universitätsmedizin Berlin- Institute of Pathology, Laboratory of Molecular Tumour Pathology and Systems Biology, Berlin, Germany; 2Lineberger Comprehensive Cancer Center- University of North Carolina, Department of Biochemistry and Biophysics- Department of Pharmacy, Chapel Hill- NC, USA; 3University Medical Center Groningen, Laboratory of Pediatrics- Section Systems Medicine of Metabolism and Signaling, Groningen, The Netherlands; 4Humboldt-Universität zu Berlin, IRI Life Sciences and Institute of Theoretical Biology, Berlin, Germany; 5DKTK-German Consortium for Translational Cancer Research- Partner Site Berlin, DKFZ- German Cancer Research Center, Berlin, Germany; 6Luxembourg Institute of Health- Proteome and Genome Research Unit, Department of Oncology, Luxembourg, Luxembourg; 7School of Medicine and Health Sciences- Carl von Ossietzky- University Oldenburg, Department for Neurosciences, Oldenburg, Germany

Introduction Oncogenic KRAS is widely acknowledged as a critical determinant of the therapeutic response of colorectal cancer (CRC) – a fact that to date is pivotal for defining an appropriate treatment strategy. Whether this also extends beyond RTK- and MAPK- targeted molecular cancer therapies, to a novel and promising class of anti-cancer drugs, namely HDAC inhibitors (HDACi), is an aspect that until now has remained largely undefined. Our aim is, therefore, to establish whether RAS is an effective predictor of response to HDACi in CRC. Ultimately, we intend to shed light on the cause underlying the limited clinical benefits of HDACi as a treatment option for solid tumours, including CRC and thereby identify opportunities to improve the prospects of HDACi treatment.

Material and methods We investigated the presence of an oncogenic-RAS dependency against a wide range of different HDAC inhibitors using model systems with intrinsic and conditionally active RAS oncogenes.
Results and discussions We uncovered an oncogenic RAS-dependent ‘safeguard’ mechanism imposed in order to evade the cytotoxic effect of HDACi and thereby apoptosis. Cells harbouring oncogenic RAS were observed to undergo a reversible senescence-like growth arrest in G2 phase, allowing for re-entry into cell cycle following a withdrawal of the inhibitor. This mechanism is implemented as a consequence of the inhibition of the RAS deacetylase, namely HDAC2, which in turn result in the generation of (hyper)acetylated RAS with increased binding affinity to BRAF and CRAF. This translates to a further amplification in MAPK-signalling and thus an increase in the priming of c-MYC for ubiquitin-mediated proteasomal degradation, thereby enabling the cells to exit the cell cycle and enter the defined protective state of G2 arrest. The prospect of HDACi treatment was effectively improved using current MAPK-targeted therapy and senolytic drugs by effectively preventing the observed pro-oncogenic effect of the HDACi treatment alone.

Conclusion Our study reveals an oncogenic RAS-dependent resistance mechanism, enabling cells harbouring oncogenic RAS, to establish a favourable cellular state of prolonged pharmacological hideout - a phenomenon that is replicated in patient-derived 3D cell culture models of CRC. This highlights the potential clinical relevance of our findings and thus the importance of a rational mechanism-based combinatorial therapeutic design in order to realise the true therapeutic potential of HDACi.

### PO-504
THE IMPACT OF KRAS MUTATIONS ON THE CYTOTOXIC EFFECTS OF AFATINIB AND ALLITINIB IN NON- small-CELL LUNG CANCER CELL LINE

**1** RJ Silva Oliveira, **1 M Eliseo Melendez, **1 N Faria Gomes, **1 AC Carloni, **1 C Carolina Munari, **1 A Lopes Carvalho, **1 R Manuel Reis. **2 Barretos Cancer Hospital; Molecular Oncology Research Center, Barretos, Brazil; **3 Life and Health Sciences Research Institute, University of Minho, Barretos, Brazil

**Introduction** EGFR alterations (overexpression and mutations) are frequent events in non-small cell lung cancer (NSCLC). In the last years, second-generation EGFR-targeted therapies, such as afatinib such as allitinib were designed, having a potent irreversible inhibitor action of EGFR and other ErbB family members. Besides the EGFR mutation status, there are no predictive biomarkers of response to this new generation of inhibitors. On this context, the aim of our study was to compare the cytotoxic effects of two irreversible anti-EGFR inhibitors in a panel of NSCLC cell line and assess the impact of KRAS mutations in the response to these agents.

**Material and methods** Total of 15 NSCLC cell lines were used. Cytotoxicity was assessed by (MTS). According to GI50 score, cell lines were classified into three groups: highly sensitive (HS), moderate sensitive (MS) and resistant (R). Muta- tional status of EGFR, KRAS, BRAF and PIK3CA was determined by direct sequencing. Sensitive H292 cell line was transfected with KRAS mutations (p.G12D and p.G12S), then profile of MAPK phospho-protein was assessed by RPPA. Subsequently, cytotoxicity, colony formation, migration and invasion were measured. In vivo chorioallantoic membrane assay (CAM) was used to evaluate the impact of KRAS mutations on tumour proliferation.

### PO-505
TARGETING PIM KINASE TO OVERCOME RESISTANCE TO PI3K-mTOR INHIBITION IN NSCLC

**1** K Gately*, **2 S Heavey, **3 S Cuffe, **4 S Finn, **5 K O’Byrne, **6 M O’Neill, **7 G Moore. **1 Trinity Translational Medicine Institute; St. James’s Hospital, Clinical Medicine, Dublin, Ireland; **2 University College London, Research Department for Tissue and Energy, London, UK; **3 Trinity College Dublin; St. James’s Hospital Dublin, Clinical Medicine, Dublin, Ireland; **4 Queensland University of Technology, Translational Research Institute, Brisbane, Australia; **5 Inflection Biosciences Ltd, Blackrock, Dublin, Ireland

**Introduction** Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality globally, having a 5 year survival rate of less than 15%, PI3K-mTOR signalling has been implicated in various hallmarks of cancer and is frequently dysregulated in NSCLC. Efforts to therapeutically target the PI3K-mTOR pathway have been hampered by the inevitable emergence of drug resistance inhibiting a durable response to treatment. Our group developed NSCLC cell line models of acquired resistance to PI3K-mTOR inhibitor GDC-0980. Resistant cells (H1975GR) were also less sensitive to PI3K-mTOR dual targeting inhibitor, BEZ235 compared to matched parent cells (H1975P) making them an ideal model to identify and interrogate drug resistance mechanisms.

**Material and methods** The sensitivity of GDC-0980 resistant cells (H1975GR) versus age-matched parent cells (H1975P) to BEZ235 following a 72 hour treatment was compared using a Cell Titre Blue cell viability assay (n=3). Alterations to the mRNA expression profile of H1975GR versus H1975P were examined using an IL-6/STAT3 signalling-specific RT2 gene profiler array (n=1). Selected genes from the array were validated by SYBR-based qPCR, immunofluorescence (IF) and western blot analysis (n=3–4). 11 miRNAs (regulated by or regulators of c-Myc/PIM) plus housekeeping control miRNAs were quantified in the H1975P/GR model by QPCR. IC50 values of pan-PIM kinase inhibitors AZD1208, IBL101 and novel PI3K/PI3K/mTOR/PIM inhibitor BBL301 inhibiting growth in H1975P versus H1975GR were compared using the BrdU assay. The effect of these drugs on RTK and c-Myc expression were examined in H1975GR by IF and western blot analysis.

**Results and discussions** In-depth characterisation of the H1975GR cells identified activation of several receptor