Material and methods The effect of NHI-Glc-2 on cell growth is tested in our primary PDAC cancer cell cultures, characterised for their hypoxic signature and LDH-A/GLUT-1 expression levels by next-generation sequencing. Inhibition of cell and tumour growth was evaluated by the SRB assay, 3D spheroid-cultures and with an orthotopic bioluminescent in vivo model. Additionally, LDH-A enzyme activity inhibition and the effect on the glycolytic rate by NHI-Glc-2 were assessed by spectrophotometry and with the Seahorse XF analyzer, respectively.

Results and discussions NHI-Glc-2 is capable of inhibiting PDAC cell growth in, especially in hypoxia, in nanomolar range and shows a synergistic effect with gemcitabine. In 3D cultures NHI-Glc-2 disrupts spheroid integrity, and preliminary in vivo studies show promising results.

Conclusion Lactate dehydrogenase A is a viable target in PDAC, and the novel LDH-A inhibitor showed improved pharmacological effect in normoxic and hypoxic PDAC cells compared to NHI-1 and NHI-2. Moreover, this compound displays a synergistic cytotoxic activity with gemcitabine, offering an innovative tool in hypoxic tumours.

PO-043 DEVELOPMENT OF TWO NOVEL MONOCLONAL ANTIBODIES AGAINST OVEREXPRESSED ANTIGENS ON PANCREATIC CANCER CELLS FOR USE IN DIAGNOSIS AND THERAPY

Introduction Pancreatic cancer is one of the deadliest cancer types with very poor survival rates and limited treatment options. Therefore, novel treatments are urgently needed. Monoclonal antibody (mAb) technology is an excellent tool for the discovery of overexpressed cell surface tumour antigens and development of mAb-based products for use in diagnosis and treatment of cancer. While several mAbs have been approved for the treatment of a wide range of cancers, none have been approved for pancreatic cancer yet. The aim of our study was to develop novel mAbs against overexpressed cell surface antigens on pancreatic cancer cells for use in cancer diagnosis and therapy.

Material and methods Novel mAbs were generated against CFPAC-1 cells using hybridoma technology and those directed against overexpressed cell surface antigens were selected and purified by affinity chromatography. Further characterisation was performed by ELISA, flow cytometry, cell proliferation and migration assays, internalisation studies, immunoprecipitation and mass spectrometry, immunohistochemistry and Western blotting.

Results and discussions We developed two novel mouse mAbs named KU44.13A and KU44.22B that were found to target CD26 and integrin alpha-3 respectively. Integrin alpha-3 was found to be widely overexpressed in human pancreatic cancer cell lines by ELISA and flow cytometry. Treatment with mAb KU44.22B induced receptor downregulation and internalisation and inhibited the growth in vitro of the human pancreatic cancer cell line Capan-2 with an IC50 of 4.5 nM. Paradoxically, treatment with this antibody increased the migration of BxPC-3 and CFPAC-1 cancer cells. CD26 expression, in turn, was limited to pancreatic cancer cell lines derived from ascites (HPAF-II and AsPC-1). Treatment with targeting mAb KU44.13A did not have any effect on cell proliferation, migration or receptor downregulation and internalisation. While neither of the two mAbs immunodetected the target antigen by Western blot, they were useful for immunohistochemical detection of the target antigens in formalin-fixed paraffin-embedded tumour sections.

Conclusion We believe these two novel mAbs are useful tools for investigating the relative expression, prognostic significance and predictive value of CD26 and integrin alpha-3 in patients with pancreatic cancer. Further studies are warranted to elucidate the therapeutic potential of these novel mAbs including their humanised or conjugated versions, in patients pancreatic and other types of cancer.

PO-044 DEVELOPMENT OF FLOW CYTOMETRIC ASSAYS FOR CAR T CELL MANUFACTURING AND PATIENT IMMUNOMONITORING

Introduction Adoptive cell therapy using genetically engineered chimeric antigen receptor (CAR) T cells has demonstrated unprecedented potency in B cell malignancies, and offers new hope for curative responses in patients suffering from cancer. However, the manufacturing process for CAR T cells is very complex and has extensive demands on personnel and infrastructure, which is a major obstacle for their routine clinical use. To overcome these hurdles, the CliniMACS Prodigy allows generation of CAR T cells in a single automated and closed system.

Material and methods CliniMACS Prodigy
MACSQuant Analyzer
MACS Antibodies

Results and discussions For assessment of CAR T cells during cell manufacturing and patient immunomonitoring we developed a set of different flow cytometric assays. These assays will be used for 1) in-process control, QC release testing, and concomitant research during the manufacturing process, and 2) for determination of CAR T cell persistence and phenotyping during patient immunomonitoring. Among others these assays allow to determine the general immune cell composition, CAR transduction efficiency, and further functional CAR T cell phenotypes like differentiation, activation, or exhaustion status.

For identification of CAR T cells we developed CAR detection reagents that specifically bind to the antigen-recognition domain of the receptor. Thus, these detection reagents discriminate between various CAR constructs, and can be used for enumeration of CAR T cells during manufacturing and immunomonitoring.

For all flow assays mentioned above so-called Express Modes have been programmed, that allow an automated acquisition and analysis of stained samples on MACSQuant Analyzers. These Express Modes feature predefined experiment settings and analysis templates, and apply a fully automated gating strategy that adapts for each individual data file. This
allows for a high standardisation by reducing operator variability, full reproducibility of data analysis, and future integration into automated workflows.

**Conclusion** Elaborate flow assays specifically designed for CAR T cells, run with high-quality antibodies and fully automated flow analysis, provide a robust assessment of cell manufacturing and patient immunomonitoring. This will help with establishing complex individualised therapies and will allow us to understand from future clinical trials in greater detail the phenotypic changes occurring throughout the life time of a CAR T cell.

### Personalised Medicine

**PO-045 EVALUATING LIQUID BIOPSIES FOR METHYLOMIC PROFILING OF PROSTATE CANCER**

1. Silva*, 1B Moran, 2C Fahy, 1V Jainic, 1M Brennan, 1W Gallagher, 1A Perry, 1University College Dublin, Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland; 2Trinity College Dublin, Institute of Molecular Medicine, Dublin, Ireland; 3St. James’s Hospital, Department of Histopathology, Dublin, Ireland

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**Introduction** Epigenetic modifications, particularly DNA methylation, are centrally involved in prostate cancer (PCa) initiation and progression. Yet, how these alterations unfold and interplay in the progression to the lethal castration resistant phenotype is poorly understood. One reason for this is the difficulty in accessing metastatic tumour deposits for study. Recently, the analysis of liquid biopsies has emerged as a useful and minimally invasive method to study tumour characteristics. The aim of this study is to explore and compare how accurately the DNA methylation patterns of liquid biopsies reflect those of the primary tumour.

**Material and methods** We identified 4 metastatic treatment-naïve PCa patients for whom matched biopsy cores (tumour and histologically matched normal), pre-biopsy urine (≤50 ml), and peripheral blood plasma (3 ml) were available. DNA was isolated from all sample types and quantified using the Qubit Fluorometer. DNA methylation was profiled using the Infinium MethylationEPIC BeadChip (Illumina), and analysed using RnBeads software. Absolute β-values were used to filter the data into probes of interest, with cut-offs for hyper- and hypo-methylation of >0.8, <0.2, respectively.

**Results and discussions** We first considered whether matched normal tissue was epigenetically distinct from tumour by comparing the methylation patterns of several genes (i.e. RARB), for which hypermethylation is considered a hallmark of PCa. Focusing next on the methylation extremes (β >0.8 or β <0.2), we observed that hypermethylation was consistently more prevalent than hypomethylation in both tissue and liquid biopsies. Enumerating these methylation extreme probes revealed that the liquid biopsies contained a higher absolute abundance of hypo- and hypermethylation than the tissue biopsies. We also found that we could detect more hyper- and hypomethylated tumour-specific probes in urine than in plasma (80% vs. 62% and 69% vs. 64%, respectively).

**Conclusion** Liquid biopsies are excellent surrogates for profiling tumour-specific DNA methylation, with urine demonstrating superior sensitivity over blood. Further analysis of differentially methylated regions in the liquid and tissue biopsies, and their relevance is PCa biology, is underway.

**PO-046 DUAL INHIBITION OF JAK AND SRC: A NOVEL AND PROMISING THERAPEUTIC COMBINATION FOR PANCREATIC CANCER**

1A Parke*, 1A Steirmann, 1D Froio, 1A Drury, 1N Vogel, 1K Murphy, 1N Deng, 1A Gill, 1P Timpson, 1M Pajic, 1The Garvan Institute of Medical Research, Cancer, Sydney, Australia; 2Royal North Shore Hospital, Department of Anatomical Pathology, Sydney, Australia

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**Introduction** Pancreatic cancer (PC) has a 5 year survival of only 6%, and persists as the 4th most common cause of cancer-related death in Western societies. A more tailored treatment approach may be beneficial as the current standard-of-care therapies offer only a modest increase in overall patient survival patient overall. Recent large-scale genomic studies have revealed that the Src/JAK/STAT3 signalling pathway is deregulated in up to 35% of PC, and is yet to be systematically examined in this disease. Consequently, we hypothesised that targeting pancreatic tumours with activated JAK/STAT3 signalling with selective JAK1/JAK2 or JAK3 inhibitors and an Src inhibitor represents a promising novel therapeutic strategy for this disease.

**Material and methods** We utilised well-annotated patient-derived cell-line models (ICGC), along with cell-lines generated from the aggressive the KPC mouse model. Using these pre-clinical models we assessed the in vitro efficacy of therapeutic strategies involving Src/JAK/STAT3 inhibition, using cell proliferation assays, 2D-drug synergy screens, and 3D organotypic invasion assays. Extracellular matrix integrity post-treatment was assessed using second-harmonic generation (SHG) imaging and picrosirius staining. To examine in vivo efficacy, we utilised a syngeneic KPC mouse model, and performed both orthotopic and subcutaneous studies.

**Results and discussions** We show that selected JAK and Src-inhibitors inhibit cell proliferation in candidate PDCLs and KPC lines, characterised by activated Src/JAK/STAT3 signalling, with combination therapy being synergistic in the majority of these cell-lines. Cell invasion was significantly inhibited in organotypic matrices, and there was decreased collagen contractility, and reduced fibrillar collagen coverage. We also demonstrated the in vivo efficacy of these therapies, and show their ability to reduce regulatory T-cells, MDSCs and tumour-associated macrophages.

**Conclusion** Our findings demonstrate the potential for tailored therapeutic strategies involving Src/JAK/STAT3 inhibition in PC, and suggest that therapeutic efficacy may be the result of targeting both tumour cells and the tumour microenvironment, as well as by overcoming tumour-induced immunosuppression.

**PO-047 ETARGET: A DIGITAL SCIENCE SOLUTION TO INTEGRATE CLINICAL AND GENOMIC DATA FOR THE MANCHESTER MOLECULAR TUMOUR BOARD (MTB)**

1J Stevenson*, 1M Ayub, 2S Dransfield, 3E Shing, 3D Barley, 3R Dunne, 4M Westaway, 1,3D Landers, 2,3M Krebs. 1Cancer Research UK Manchester Institute, Clinical and Experimental Pharmacology, Manchester, UK; 2Christie NHS Foundation Trust, Experimental Cancer Medicine, Manchester, UK; 3Christie NHS Foundation Trust, Biotbank, Manchester, UK; 4Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, Manchester, UK; 5The University of Manchester, Research IT, Manchester, UK; 6Microsoft Limited, Customer Success Unit, Manchester, UK; 7AstraZeneca, Early Clinical Development, Cambridge, UK; 8University of Manchester, Division of Cancer Sciences, Manchester, UK

10.1136/esmoopen-2018-EACR25.580

**Introduction** Pancreatic Cancer (PC) is a 5 year survival of only 6%, and persists as the 4th most common cause of cancer-related death in Western societies. A more tailored treatment approach may be beneficial as the current standard-of-care therapies offer only a modest increase in overall patient survival. Recent large-scale genomic studies have revealed that the Src/JAK/STAT3 signalling pathway is deregulated in up to 35% of PC, and is yet to be systematically examined in this disease. Consequently, we hypothesised that targeting pancreatic tumours with activated JAK/STAT3 signalling with selective JAK1/JAK2 or JAK3 inhibitors and an Src inhibitor represents a promising novel therapeutic strategy for this disease.

**Material and methods** We utilised well-annotated patient-derived cell-line models (ICGC), along with cell-lines generated from the aggressive the KPC mouse model. Using these pre-clinical models we assessed the in vitro efficacy of therapeutic strategies involving Src/JAK/STAT3 inhibition, using cell proliferation assays, 2D-drug synergy screens, and 3D organotypic invasion assays. Extracellular matrix integrity post-treatment was assessed using second-harmonic generation (SHG) imaging and picrosirius staining. To examine in vivo efficacy, we utilised a syngeneic KPC mouse model, and performed both orthotopic and subcutaneous studies.

**Results and discussions** We show that selected JAK and Src-inhibitors inhibit cell proliferation in candidate PDCLs and KPC lines, characterised by activated Src/JAK/STAT3 signalling, with combination therapy being synergistic in the majority of these cell-lines. Cell invasion was significantly inhibited in organotypic matrices, and there was decreased collagen contractility, and reduced fibrillar collagen coverage. We also demonstrated the in vivo efficacy of these therapies, and show their ability to reduce regulatory T-cells, MDSCs and tumour-associated macrophages.

**Conclusion** Our findings demonstrate the potential for tailored therapeutic strategies involving Src/JAK/STAT3 inhibition in PC, and suggest that therapeutic efficacy may be the result of targeting both tumour cells and the tumour microenvironment, as well as by overcoming tumour-induced immunosuppression.