transformed cells that occasionally appear in the body; on the other hand, cancer cells can recruit immune cells endowed with pro-tumorigenic activity.

Our lab previously developed a strategy for targeted gene-based delivery of interferon alpha (IFNa) to tumours by tumour infiltrating monocytes/macrophages, which induces robust anti-cancer responses in several experimental models without inducing strong IFN responses in normal tissues as compared to systemic administration of recombinant IFNa. Whereas a sustained output could ensure long-term protection from tumour recurrence, it may raise concerns for long-term side effects, especially in case of cancer eradication.To overcome this issue, we are developing inducible strategies to control the amount of IFNa secreted in the tumour microenvironment.

Material and methods By fusing a destabilising domain (DD) to a protein of interest (POI) the former can confer its instability to the latter. This destabilisation can be rescued in a reversible and dose dependent manner with the addition of a small molecule specifically binding to the DD. To apply this technology to our strategy we have designed and *in vitro* tested different fusion proteins of IFNa (DD-IFNa). We also developed improved DD-IFNa with the addition of flexible and/or cleavable linkers and selected them for their capacity to be stabilised in a dose dependent manner in presence of their specific ligand *in vitro*.

Results and discussions Through this approach, we have identified effective fusion proteins with low basal activity and high fold induction upon ligand treatment. These novel tunable forms of IFNa are functional and their specific activity are comparable to the wild type cytokine in inducing IFN responsive genes.

Based on these promising *in vitro* results we are now translating these new platforms *in vivo* to test their efficacy in inducing anti-tumour responses in melanoma, colon and glioma models of cancer.

Conclusion In the perspective of clinical translation our approach can be used in the future to switch on/off the levels of IFNa in a tunable and personalised fashion for cancer eradication.

PO-041 COLD ATMOSPHERIC PLASMA: A POTENTIALLY SELECTIVE AND NON-INFLAMMATORY ANTI-CANCER THERAPY

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Introduction Considering the increased need for alternatives to current treatments, a new therapy based on plasma, the fourth state of matter, has recently raised the medical community's attention. The aim of this work was to evaluate the effect, selectivity and mechanisms of action of cold atmospheric plasma (CAP) in a human retinoblastoma cell line.

Material and methods An electronic device was designed to generate CAP, in open air above multiwell plates where Y79 cell cultures were seeded. Plasma emission spectrum was captured by a spectrometer. In order to evaluate the cytotoxicity and selectivity of CAP, metabolic activity of similarly treated Y79 and human fibroblasts HFF1 cells was measured. Apoptosis detection, analysis of mitochondrial membrane potential (MMP) and cell morphology were studied to determine the type of cell death. Propidium-iodide/RNAse staining was used to study the cell cycle and genotoxic effects were assessed by comet assay. Oxygen and nitrogen reactive species (RS) and oxidative defenses were measured. In order to explore the interaction of the electric field with voltage-gated calcium channels, blockade with verapamil was applied. Clonogenic assay screened for long term survival.

Results and discussions After 60 s of CAP treatment, the metabolic activity of Y79 cells decreased more than 50%, mostly due to apoptosis, while HFF1 endured viable. Cell survival was shortened. Accumulation of Y79 cells in S and G2/M phases was recorded, nevertheless, no DNA strand breaks were detected. Plasma emission spectrum displayed several peaks in ultraviolet domain. Concerning RS, the concentration of intracellular peroxides and nitric oxide was increased. However, antioxidative defenses were not triggered and reactive oxygen species inhibitors were not capable of abrogating cytotoxic effects of CAP. Similarly, verapamil did not protect cells from death.

Conclusion This study suggests a potential novel therapy based on plasma able to selectively target tumour cells while preserving the non-inflammatory environment.

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PO-042 TARGETING HYPOXIC PANCREATIC CANCER CELLS WITH GLUCOSE CONJUGATED LACTATE DEHYDROGENASE INHIBITOR NHI-GLC-2

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Introduction Pancreatic ductal adenocarcinoma (PDAC) is an abysmal disease with a 5 year survival rate of merely 8%. The tumour microenvironment is one of the factors contributing to PDAC chemoresistance. More specifically, the hypoxic tumour cores and the metabolic switch to aerobic glycolysis (e.g. the Warburg effect), contribute to the lack of drug response. Interestingly, two glycolysis components glucose transporter 1 (GLUT-1) and lactate dehydrogenase A (LDH-A) are overexpressed in PDAC. The latter, LDH-A, is also correlated with prognosis in metastatic PDAC.

N-Hydroxyindole-based LDH-A inhibitors (NHI-1 and NHI-2) have shown a synergistic effect in hypoxic PDAC cells when combined with gemcitabine. A glucose conjugated NHI-Glc-2 was designed to exploit the GLUT-1 overexpression in PDAC cells and in the present study we evaluated whether this novel compound further improved the pharmacological effect of LDH-A inhibitors.

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

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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 cell lines. 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

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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 persistance 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