direct modulation of UPR signalling could sensitise head and neck squamous cell carcinoma (HNSCC) to RT3D.

Material and methods ER stress agents including novel agents targeting UPR signalling were screened for sensitisation to RT3D in a panel of HNSCC cell lines. Alterations to reovirus protein levels and viable virus particle production was assessed. A 3D tumour spheroid model was used as a more accurate method to study stress *in vitro*. Recombinant protein expression and shRNA techniques were utilised to study how the unfolded protein response impacts reovirus replication. ER stress measured by GRP78 expression was also assessed in HNSCC patient samples.

Results and discussions The PERK inhibitor GSK2606414 and the canonical ER stress agent thapsigargin were shown to sensitise HNSCC to RT3D. PERK inhibition resulted in increased viral capsid protein levels and increased viable RT3D production from target cells. PERK inhibition enhanced the ability of RT3D to eradicate HNSCC 3D tumour spheroids. PERK inhibition increased RT3D spread in 3D spheroids while perturbing normal ER chaperone levels and inhibiting RT3D induced LC3A/B. In HNSCC patient samples, GRP78 was found to be elevated in tumour vs stroma with significant variation between patients. This highlights the importance in understanding the link between ER stress levels/UPR signalling and reovirus efficacy.

Conclusion We demonstrate that modulators of the ER stress response represent a novel mechanism of sensitisation to RT3D in head and neck.

PO-032 | **DISPLACEMENT OF HEXOKINASE 2 FROM MITOCHONDRIA INDUCES MITOCHONDRIAL CA2 +OVERLOAD AND CASPASE-INDEPENDENT CELL DEATH IN CANCER CELLS**

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Introduction Hexokinase 2 (HK2) phosphorylates glucose for starting its utilisation in glycolysis and pentose phosphate pathway. In many cancer cell types these processes are enhanced and HK2 expression is strongly induced and mainly localised to the outer mitochondrial membrane, where it also exerts an anti-apoptotic activity. Genetic ablation in mouse highlights HK2 importance in tumour formation. Therefore, HK2 is a good target for antineoplastic strategies, but HK2 inhibitors can have important side effects as they affect glucose metabolism. Here we have developed an antineoplastic strategy based on HK2 detachment from mitochondria in order to induce tumour cell death without inhibiting hexokinase enzymatic activity.

Material and methods Peptide design and synthesis; hexokinase enzymatic activity assays. Measurements of mitochondrial membrane potential, intracellular Ca^{2+} levels, cell death and *in vitro* and *in vivo* tumorigenic assays on human and mouse cancer cell models (CT26 colon cancer cells, 4 T1 breast cancer cells, HeLa cervix carcinoma cells and primary human B-CLL cells).

Results and discussions We have observed that in cancer cells HK2 locates at contact sites between mitochondria and endoplasmic reticulum called MAMs (mitochondria-associated membranes). We could selectively detach HK2 from MAMs by using a peptide that does not perturb hexokinase enzymatic activity. This treatment rapidly induces opening of the Inositol-3-Phospate-Receptor and the ensuing Ca^{2+} transfer from endoplasmic reticulum to mitochondria. As a consequence, a Ca^{2+} overload occurs in mitochondria, leading to permeability transition pore opening, mitochondrial membrane depolarization and apoptosis in a caspase-independent way. Peptide administration reduces allografic growth of breast and colon cancer cells without any noxious effect on healthy tissues, and elicits death of B-cell chronic lymphocytic leukaemia (B-CLL) cells freshly obtained by patients and *in vivo*.

Conclusion We have reported that HK2 locates in MAMs of cancer cells, where it acts as an important player in the control of their survival. Targeting HK2 with a peptide-based strategy constitutes a novel and promising anti-neoplastic approach.

PO-033 | **IDENTIFICATION AND FUNCTIONAL EVALUATION OF MONOCLONAL ANTIBODIES SPECIFICALLY TARGETING HUMAN CARBONIC ANHYDRASE IX**

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Introduction Poor vascularisation of solid tumours leads to inadequate nutrient and oxygen supplies which forces tumour cells to reprogram their metabolism. As a consequence the tumour cell's environment becomes acidic and hypoxic. This, in turn, triggers signalling cascades involving for example heterodimeric hypoxia-inducible factor (HIF). Activation of this hypoxia-induced transcriptional program is crucial for the survival of tumour cells in their hostile microenvironment but also their ability to metastasize.

One of the genes upregulated through the HIF pathway is carbonic anhydrase (CA)-IX (CAIX, gene G250/MN-encoded transmembrane protein). CA-IX catalyses carbon dioxide (CO_2) thereby generating a proton (H^+) and bicarbonate (HCO_3^-), the latter of which is transported back into the cell and utilised to help safeguard intracellular pH (pHi) stability.

Except for the stomach and the gallbladder, CA-IX expression is negligible in normal tissues. In contrast, a broad range of tumours express high levels of CA-IX, where the protein can serve as a biomarker for the early stages of tumour development but also as tumour marker of hypoxia associated with resistance to chemotherapy and radiotherapy.

Material and methods Preclinical and clinical studies have shown that CA-IX is a promising therapeutic target for detection and therapy for several cancer types. To date only a limited number of ant-CAIX monoclonal antibodies (mAbs) have been available for clinical testing as therapeutic and imaging agents. In the current study, we generated and functionally categorised a panel of 51 mouse mAbs that specifically bind to human CA-IX.

Results and discussions Characterisation of the mAbs revealed that of the mAbs with the best biophysical characteristics, three[3] mAbs are suitable as an antibody-drug conjugate (ADC), two[2] mAbs inhibit the CA-IX enzyme activity, and one[1] mAb that is suitable for CA-IX imaging purposes.

Conclusion These preliminary data presented here could thus form the basis for the development of novel CA-IX targeted immunotherapies and diagnostic tools for the treatment of cancer.