Introduction Walker 256 tumour (W256) is commonly used in experimental studies to analyse beneficial effects of medicinal plants found in the Amazon as *Mucuna pruriens*. This plant possesses several properties as their use in Parkinson’s disease, general antineoplastic effects and antioxidant activity. Therefore, this experiment aims to evaluate brain tumour inhibition effect of *M. pruriens* extract in rats.

**Material and methods** *M. pruriens* seeds powder was extracted with supercritical carbon dioxide extract (SC-CO2). The right caudate nucleus was located using the following coordinates: AP = 1.5 mm; DV = 5 mm; LL = 2.5 mm. Subsequently, 7 × 10^6 tumour cells were inoculated into the animals’ brains through stereotaxic surgery. Male *Rattus norvegicus* were randomised into 3 groups (n = 5): 1. Control group (CG) – W256 inoculated rats; 2. *Mucuna* group (MG) – W256 inoculated and treated with *M. pruriens* extract (250 mg/kg); 3. Tamoxifen group (TG) – W256 inoculated and treated with Tamoxifen (20 mg/kg). Intraperitoneal administration in treated groups occurred during 7 days, on the eighth day euthanasia was performed by decapitation. After euthanasia, animals with hematoxylin/eosin. Body weights were recorded in the neoplastic cells formation and the smallest amount of congestive areas in nervous tissue. In TG an important inflammatory reaction in meninges occurred even without tumour formations. The most prevalent type of death and treated cells are predominantly in G0/G1 phase.

**Conclusion** Levels of oxidative stress and antioxidant defenses involved in the plasma effect on breast cancer cells. The results obtained encourage further studies. Funding FCT, Portugal (UID/NEU/04359/2013), FEDER-COMPETE (FCOMP-01–0124-FEDER-028417, POCI-01–0145-FEDER-007440)

Introduction Breast cancer has a high incidence rate worldwide with about 1.67 million new cases. The need for new, effective and free of side effects therapies is growing as ageing is modifying the epidemiology of cancer. Cold atmospheric plasma (CAP), known as the fourth state of matter, a gas with enough energy to ionize a significant fraction of particles, has come into attention as a potential anti-tumour therapy. Our previous studies showed that CAP determined the decrease of breast cancer cells viability after an exposure of only 60 s. The goal of this study was to evaluate the effect of cold atmospheric plasma on breast cancer cell line based on reactive oxygen species (ROS), types of cell death and cell cycle.

**Material and methods** In this study, we used a hormonal receptor positive breast cancer (MCF7). Cells were cultured, plated and exposed to CAP, for different periods of time: 60 and 120 s and, using a homemade CAP ejector. To assess ROS intracellular concentration, cell cultures were evaluated through specific probes, namely 2’,7’-dichlorodihydrofluorescein diacetate (DCFH2-DA) and dihydroethidium (DHE). The levels of glutathione antioxidant defense (GSH) were also evaluated and all studies were performed 2 and 24 hours after CAP exposure. The cell death type and cell cycle were assessed by flow cytometry using Annexin V/propidium iodide (PI) and PI, respectively. Currently, we are performing the same studies in triple negative breast cancer cell line (HCC1806).

**Results and discussions** After 2 hours of CAP therapy, ROS levels do not show any variation compared to the control. Intracellular content of superoxide anion was of (97.36 ± 11.64)% on MCF7 cell line exposed to CAP for 120 s. However, after 24 hours the superoxide anion content was (87.92 ± 21.13)% Levels of glutathione remained similar to the control. Our preliminary results suggest that apoptosis is the most prevalent type of death and treated cells are predominantly in G0/G1 phase.

**Conclusion** Levels of oxidative stress and antioxidant defenses suggest that other events besides ROS formation might be involved in the plasma effect on breast cancer cells. The results obtained encourage further studies.

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Introduction Oncolytic viruses are able to enter and selectively replicate in cancer cells. These self-propagating agents kill through multiple mechanisms and have the potential to promote anti-tumour immune responses. Reovirus strain type 3 Dearing (RT3D) is an oncolytic virus actively under clinical investigation. RT3D has been shown to induce ER stress and trigger the downstream unfolded protein response (UPR) in a variety of tumour types. Recent evidence has indicated that proteasome inhibition in multiple myeloma, and BRAF inhibition in BRAF mutant melanoma, sensitise to RT3D through perturbation of the normal cellular response to ER stress. We sought to investigate if more
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 sensitize 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.

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**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 Ca2+ 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-Phosphate-Receptor and the ensuing Ca2+ transfer from endoplasmic reticulum to mitochondria. As a consequence, a Ca2+ overload occurs in mitochondria, leading to permeability transition pore opening, mitochondrial membrane depolarization and apoptosis in a caspase-independent way. Peptide administration reduces allograftic 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.

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**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 (CO2) thereby generating a proton (H+) and bicarbonate (HCO3-), 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 anti-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, three1 mAbs are suitable as an antibody-drug conjugate (ADC), two2 mAbs inhibit the CA-IX enzyme activity, and one3 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.