to identify the potassium channels that could be implicated in the response to TNF.

**Material and methods** We analysed the effects of TNF on two CRC cell lines, HCT116 that is KRAS mutated and HT29 that is KRAS wildtype. Cell lines viability and migration were determined by MTT assay and scratch assay, respectively. Cell cycle was examined by propidium iodide DNA staining. Gene expression of TNF pathway and potassium channels was measured by RTqPCR and Western blot.

**Results and discussions** Our work shows that TNF increased the migration of HT29 cells while reducing that of HCT116. In addition, TNF reduced the viability of HCT116 cells and their colony formation capacity. Moreover, cell cycle analyses showed an increase in the proportion of sub-G1 phase in HCT116 cells after TNF treatment, with no effect on HT29 cells. We also demonstrated an increased expression and phosphorylation of STAT3 protein in HT29 cells, contrary to HCT116 cells that showed reduced phosphorylation following TNF treatment. Interestingly, exogenous TNF increased the transcriptional expression of TNF in both cell lines and this result was associated with an increased expression of its receptor, TNFR2, only in HT29 whereas it was abrogated in HCT116 cells. Furthermore, TNF caused a global decrease in the expression of potassium channels coding genes in HCT116 cells, while this effect was less pronounced in HT29 cells.

**Conclusion** Taken together, our results suggest that the modulation of TNF pathway could be associated to KRAS status. Furthermore, potassium channels could be implicated in CRC cells response to TNF.

**PO-043 TARGETING THE THIOL OXIDOREDUCTASES ERp57 AND PDI HITS CANCER CELLS ON MULTIPLE FRONTS: PROLIFERATION, RADIORESISTANCE AND ER STRESS RESPONSE (UPR)**

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**Introduction** Fast-growing tumour cells show enhanced protein synthesis and therefore depend on efficient folding for nascent export proteins in the endoplasmic reticulum (ER). Herein, the two most prominent ER resident thiol oxidoreductases ERp57 and PDI play important parts in formation of disulfide bonds in client proteins. This and the finding that both proteins fulfils various roles also in other compartments (i.e. cytoplasm, nucleus, cell membrane) encouraged us to investigate the impact of their depletion on colorectal cancer cells.

**Material and methods** Using an inducible knockdown (KD) system we tested ERp57 and PDI deficiency in long term survival assays in normoxia and hypoxia combined with irradiation.

**Results and discussions** KD of ERp57 or PDI triggered a severe attenuation of proliferation, but only ERp57 deficiency led to activation of the PERK-dependent UPR and apoptosis. When combined with an ERp57 KD, irradiation displayed the most dramatic growth reduction even under 1% oxygen. The absence of ERp57 reduced expression of cellular proliferation factors like c-Myc, PLK-1, AKT, PDK1, ERK1,2 and others. Further, we demonstrated for the first time that PDI is an essential activator of the ER stress sensor PERK that enforces cancer cell survival under global ER stress in hypoxia. In the absence of ER stress, ERp57 functions as a reductase for PDI that keeps PERK in an inactive state.

**Conclusion** Our data identified ERp57 and PDI as promising new targets for a mono- and combination anti-cancer therapy due to multiple cellular points of attack.

**PO-044 POSSIBLE INVOLVEMENT OF G2/M BLOCK DEFECTS IN CELL SENSITIVITY TOWARDS ATR INHIBITORS**

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**Introduction** Dysregulation of cell proliferation mechanisms and of DNA damage response (DDR) is a hallmark of tumorigenesis. Some mutations contribute to resistance to DNA-damaging agents, often seen in neoplastic cells, while loss of one or more DDR pathways makes cancer cells more reliant on those still functional. This opens a possibility of designing targeted therapeutics, utilising so-called synthetic lethality.

Previously, we observed that Sezary syndrome-derived cell line, SeAx, was about 50 times more sensitive towards an ATR inhibitor, VE-822, than other lymphoma/leukaemia cell lines tested. Similar, albeit less striking, tendency was observed for ATR inhibitors: VE-821 and AZD6738, but not ATM inhibitors. The aim of this study is to clear up the genetic background of the observed effect.

**Material and methods** Cutaneous T-cell lymphoma (CTCL) cell lines: SeAx, Hut78, MyLa2000 were used as preclinical models. ATR knock-down was performed by siRNA transfection. KE-822-resistant SeAx variants (0.05R and 0.08R) were induced by culturing cells with increasing VE-822 doses for at least 3 weeks. DNA repair pathways functionality was confirmed by combining genotoxic drugs with inhibitors of proteins crucial for a given pathway; in case of synergy, the pathway was regarded as functional.

**Results and discussions** • Prolonged siRNA-induced ATR knock-down slightly slows cell proliferation, but does not affect viability of SeAx cells, suggesting an off-target effect of VE-822.
• However, 0.05R and 0.08R variants are cross-resistant to all ATR inhibitors tested (irrespective of their structure) and ATR inhibitors fail to block ATM pathway, suggesting that toxicity of VE-822 is indeed due to ATR inhibition.
• Sensitivity of SeAx cells to VE-822 cannot be explained by synthetic lethality, resulting from defective DDR, since the key DNA repair pathways are functional in SeAx. 4) SeAx cells fail to activate G2/M block in response to genetic insult, and we postulate that the defective checkpoint renders them sensitive to ATR inhibitors.

**Conclusion** Checkpoint defects have previously been related to cell chemosensitivity, though not in the context of ATR inhibition. We plan to further corroborate our findings by pinpointing mutations responsible for G2/M block defects and performing a phenotypic complementation with a wild-type gene(s) copy, to confirm that the cells actually gain resistance.