major subtypes of lung cancer. NSCLC patients only have a 15% five-year survival rate. The major subtypes of NSCLC are lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). LUSC accounts for more than 40 0000 deaths each year and unlike LUAD there are limited targeted therapies. Therefore, a great deal of work still needs to be done to understand the drivers for this cancer.

Material and methods shRNA was utilised to modulate the levels of BCL11A and SOX2 in LUSC cell lines. In vitro 3D colony assays and xenograft mouse models were employed to understand the role of BCL11A in driving LUSC tumorigenesis. To explore the role of BCL11A in vivo, a Cre-inducible BCL11A overexpression mouse model was used. To further investigate BCL11A in LUSC, a mouse tracheal basal cells organoid assay was employed. CHIP-seq, immunoprecipitation and immunoblotting assays were designed to dissect the mechanism by which BCL11A elicits its function in LUSC. Drug dose response assays were used to test the efficacy of SETD8 inhibitors and cisplatin on an array of lung cancer cell lines.

Results and discussions Analysis of TCGA has revealed BCL11A to be upregulated in LUSC but not LUAD. Subsequently reducing BCL11A levels in LUSC cell lines results in diminished xenograft tumour growth. Inversely, its overexpression in vivo led to lung airway hyperplasia and the development of reserve cell hyperplastic lesions which is a precursor to squamous metaplasia. Moreover, deleting Bcl11a in mouse tracheal basal cells abolished the development of tracheosphere organoids while its overexpression led to solid tracheospheres expressing markers of squamous cells.

At the molecular level we found BCL11A to be a target of SOX2 and we show that it is required for the oncogenic role of SOX2 in LUSC. Furthermore, we showed that BCL11A and SOX2 interact at the protein level and that together they co-regulated the expression of several transcription factors. We demonstrate that pharmacological inhibition of SETD8, a gene co-regulated by BCL11A and SOX2, alone or in combination with cisplatin treatment, shows significant selectivity to LUSC in comparison to LUAD cells.

Conclusion Collectively, these results indicate that the disruption of the BCL11A-SOX2 transcriptional program provides a future framework for the development of targeted therapeutic intervention for LUSC patients.

PO-087  OXIDATIVE STRESS AS A SELECTIVE ANTICANCER AGENT: PRECLINICAL EVALUATION OF A TARGETED COMBINATION STRATEGY FOR MUTANT P53 NON-SMALL CELL LUNG CANCER

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Introduction Increased oxidative stress is a hallmark of cancer cells, which makes them more vulnerable to induction of reactive oxygen species (ROS). P53 plays a crucial role in sensing and removing oxidative damage to DNA, and inactivating mutations in the TP53 gene attenuate this function. In addition, it was shown that mutant p53 is able to suppress the function of major antioxidant factors. Therefore, mutant p53 renders cancer cells even more susceptible to the induction of oxidative stress. Besides p53, the poly (ADP-ribose) polymerase 1 (PARP-1) protein plays an important role in the repair of ROS-induced DNA-damage. This led us to explore the potential of combining oxidative stress induction with the targeted inhibition of the PARP-1 protein to selectively target mutant p53 NSCLC cancer cells.

Material and methods APR-246 and Auranofin (inhibition glutathione (GSH) and/or thioredoxin reductase 1 (TrxR1)) and Olaparib (PARP-1 inhibitor) were used. The cytotoxicity (SRB-assay) of these compounds was determined in a panel of NSCLC cell lines with different p53 status, including isogenic cell lines (p53 shRNA-knockdown, p53 knock-in). Total GSH content (GSH/GSSG-GloTM) and ROS content (CellROX) were determined. N-acetyl-l-cysteine (NAC) was used as a potent ROS-scavenger. Induction of apoptosis/cell death was determined by the Annexin V/PI assay (FC) or the Cytotox Reagent (IncuCyte). DNA-damage was assessed by g-H2AX foci (IF) and the Comet Assay. Synergism was determined using the Additive model.

Results and discussions P53Mut knock-down reduces the cytotoxic effect of APR-246, Auranofin and Olaparib, while p53Mut knock-in sensitised cells for all three compounds. APR-246/Olaparib treatment reduced GSH levels and increased ROS content, resulting in a strong accumulation of DNA-damage and synergistic induction of cell death. Co-treatment with NAC or p53-knockdown significantly reduced this cytotoxic response. Similar synergistic effects were observed for Auranofin/Olaparib treatment in several cell lines with clinically relevant p53 mutations.

Conclusion Mutant p53 protein expression renders NSCLC cells more susceptible to APR-246, Auranofin and Olaparib treatment. In addition, the combination of oxidative stress induction (APR-246, Auranofin) with PARP-1 inhibition (Olaparib) results in remarkable synergistic effects in the presence of mutant p53. Therefore, this combination strategy could be a promising and selective treatment option for mutant p53 NSCLC patients in which resistance to standard therapies often occurs.

PO-088  ABSTRACT WITHDRAWN

PO-089  135 TARGETING TREATMENT RESISTANCE IN BREAST CANCER SUBTYPES VIA LMTK3 INHIBITION

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Introduction LMTK3 is an oncogenic Receptor Tyrosine Kinase (RTK) implicated in several types of cancer including breast, lung, gastric and colorectal. Numerous mechanistic and translational data describe the role of LMTK3 predominantly in breast cancer and support investigating LMTK3 as a potential new therapeutic target in this particular tumour type. This study therefore aims to develop selective LMTK3 inhibitors.

Material and methods The Bellbrook Laboratories Transcreeener assay kit was employed and 30 000 compounds were screened to detect novel LMTK3 inhibitors. Nearly 100 of them significantly inhibited LMTK3 activity and were therefore chosen for 10-point concentration-response profiling in duplicate and LC-MS analysis. The top 50 test compounds were clustered
Results and discussions Two (C28 and C36) out of the 30,000 compounds that were screened inhibited by >95% the activity of only 10 and 8 kinases respectively. Moreover, the S(35) selectivity index of C28 was 0.186 while the selectivity index of C36 was 0.114. Interestingly, quantitative analysis of 38 kinase inhibitors currently used in clinical oncology showed a comparably low S(35) score as C28 and C36. It is expected that the crystallisation of the LMTK3 kinase domain that is currently being conducted as well as co-crystallisation experiments with these inhibitors and other analogues will guide a rational optimisation strategy of each chemotype. These findings will then be validated by in vitro kinase assays and in a cellular context.

Conclusion More work is required; however, these data represent a step towards the development of the first LMTK3 inhibitors that may have potential for broad clinical utility in breast cancer.

Introduction Cocaine- and amphetamine-regulated transcript (CART) peptides are neuropeptides involved in regulating physiological processes, such as feeding and drug reward. Recent studies have associated high CART expression with worse overall survival in patients with small-bowel carcinoid tumours and oestrogen receptor-positive (ER+) breast cancer patients, suggesting that this negative impact on survival is potentially related to ERα.

Results and discussion A56 CART was also shown to be associated with poor OS or DMFS in a cohort of ER-/+ breast cancer patients. Intriguingly, SMARCD1 expression did not correlate with poor OS or DMFS in a cohort of ER+ breast cancer patients, suggesting that this negative impact on survival is potentially related to ERα.

Conclusion In conclusion, we suggest that CART expression results in the recruitment of chromatin remodelling complexes to ERα in order to facilitate the regulation of receptor function.