oncoprotein itself has proven difficult, and attempts to target downstream effectors have been hampered by the activation of compensatory resistance mechanisms (Bernards et al., Cell 2012). Notably, while MEK inhibition initially leads to ERK inactivation, P-ERK levels are quickly restored thanks to the upregulation of multiple growth factors receptors (Sun et al., Cell Reports 2014). Here we explored the possibility of targeting PTPN11 as a key mediator of receptor tyrosine kinase signalling in order to overcome intrinsic resistance to MEK inhibition.

**Material and methods** Using a panel of 6 KRAS mutant NSCLC cell lines, we studied the biochemical and cell proliferation effect of combining the PTPN11 inhibitor SHP099 (Chen et al., Nature 2016) with the MEK inhibitor AZD6244. We confirmed our findings in CRISPR-based PTPN11 knock-out clones of H2122 and H1944 cells. Importantly, we studied the effect of SHP099 in vivo in xenograft and PDX models, as well as in KRAS^{G12D,G12D} p53^{+/−} genetically engineered mouse model of NSCLC. To gain mechanistic insights into the effect of PTPN11 inhibition on mutant RAS activity, we used a panel of isogenic Rasless murine embryonic fibroblasts (Esposito et al., Semin Cancer Biol. 2018; NCi RAS Initiative) expressing human wild-type or mutant (G13D, G12C, G12D, G12V or Q61R) KRAS genes.

**Results and discussions** Our data show that depletion or inhibition of PTPN11 in KRAS mutant NSCLC cell lines invariably induces sensitivity to AZD6244. Surprisingly, in vivo models suggest that targeting PTPN11 is by itself sufficient to impair tumour growth, mainly due decreased RAS-GTP loading and induction of cellular senescence under growth factor-limiting conditions. Of note, our data suggest a correlation between the residual GTP hydrolysis capacity of the distinct KRAS mutants and their responsiveness to upstream regulators like PTPN11.

**Conclusion** While in the past efforts have been focused on targeting KRAS downstream effectors, our data suggest that inhibiting upstream mediators like PTPN11 could be effective either alone or in combination with downstream inhibition. Our results suggest that targeting PTPN11 could be of clinical utility especially for tumours driven by KRAS mutants with high residual GTP hydrolysis capacity.

**New Therapies**

**PO-020**

**DISCREPANCY IN EFFICACY OF DISULFIRAM BETWEEN NUP98-PHF23 FUSION ACUTE MYELOGENOUS LEUKAEMIA CELL LINE AND IN VIVO MOUSE MODEL: SHARING NORMAL HEMATOPOIETIC STEM CELLS NICHE**

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**Introduction** NUP98 has numerous partner genes of which plant homeodomain (PHD) finger protein 23 (PHF23) fusion with NUP98 (NP23) can be detected by RT-PCR in patients with cytogenetically normal acute myelogenous leukaemia (AML). In this fusion transcript of NP23 plant homeodomain (PHD) of PHF23 is known to specifically bind H3K4me3 residues and act as chromatic modifier. Disulfiram (DSF) which inhibit the binding of PHD to H3K4me3 residues was selectively killed NP23 myeloblasts in vitro and therefore, we planted to evaluate the efficacy of DSF in vivo.

**Material and methods** Cultured 961 C cells (CD45.2), NP23 myeloblast cell were transplanted in B57BL/6 mice (CD45.1) (figure1). Using limit dilution assay the number of leukemic stem cells (LSCs) can be calculated. Certain quantity of 961C were transplanted in B57BL/6 mice and DSF was treated after 1 week. The engraftment level was monitored with CD45.2. The Kaplan Meier survival curve was plotted and compared the survival between therapeutic groups and control.

**Results and discussions** 961 C cells could be transplanted without radiation in recipient mice. Calculated LSC was estimated to be 1 out of 184 cells (95% CI range, 56–609). When treated with DSF (several dosage and several administrative route) in 961C recipient mice we could not show any survival benefit between groups even though engraftment level was consistent in both group.

**Conclusion** We could not show the survival advantage of DSF in 961C transplanted immunocompetent mouse but could know that 961 C cells shared niche with normal hematopoietic stem cells (HSCs). We expect that 961 C cells and their transplanted mice will be used as in vivo system for the new drugs development as well as for the basic study dealing with niche for normal HSCs and LSCs.

**PO-021**

**INHIBITION OF MK2 STABILISES WILD-TYPE P53 AND IMPROVES TEMOZOLOMIDE EFFICACY IN GLIOBLASTOMA**

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**Introduction** Temozolomide (TMZ) remains the first line therapy for glioblastoma since its approval in 2005. TMZ efficacy is marginal as patients succumb to the disease within two years, urging the need for new therapeutic approaches. Frequent genetic alterations in the DNA damage machinery of most cancer cells opens a window of possible therapeutic vulnerability. Tumours lacking p53 rely heavily on the G2/M cell cycle checkpoint for DNA repair, due to the loss of a functional G1 checkpoint. Therefore, inhibiting the G2/M checkpoint allows for synthetic lethality, as these cells are forced to continue through the cycle with unrepaired DNA ultimately resulting in cell death. It was previously reported that in the absence of p53, cells depend on the checkpoint kinase MK2 for cell cycle arrest and survival after DNA damage by doxorubicin. However, it is currently unknown if targeting MK2 is a viable therapeutic approach for glioblastoma therapy in conjunction with TMZ. Thus, we aimed to investigate the effect of inhibiting MK2 and whether it will improve the effectiveness of TMZ in glioblastoma.

**Material and methods** Inhibition of MK2 was conducted using siRNA, CRISPR-Cas9 and pharmacological inhibitors. Signalling pathways, stability and nuclear retention of proteins was investigated whether it will improve the effectiveness of TMZ in glioblastoma.
Introduction
Sodium iodide symporter (hNIS) expression is reported across all subtypes of breast cancer (BC) and is a potential endogenous gene target for radioiodine therapy [PlosOne8(1: e54055, 2012]. Successful clinical application however relies on very high expression of functional NIS in BC cells. We have been exploiting various transcriptional regulation approaches for endogenous NIS expression in BC [Scientific Reports6:19341, 2016]. Here, we show that differential induction of NIS expression in BC is possible using Benzaamide class of HDACi (bHDACi) with minimal off-target effects on other cell types.

Material and methods
The effect of bHDACi, i.e. CI-994, Chidamide, MS-275 and AR-42, on NIS gene transcription was verified on a panel of human cancer cells by RT-PCR. Functional assessment was done by non-radioactive iodine uptake. Further, ZR-75 pre-treated orthotopic tumour showed increased NIS expression. In comparison to untreated and only I\textsubscript{131} treated group, a combination treatment of AR-42 with 1mCi I\textsubscript{131}, BLI imaging showed 20% and 30% signal drop on day1 and day 2 respectively indicating specific radio-ablation effect.

Conclusion
The combination of CI-994 inhibition alongside TMZ provides a new therapeutic strategy to enhance the effectiveness of current chemotherapy in p53 proficient tumours.

Results and discussions
Our data demonstrates that inhibition of MK2 increases the stability and expression of p53, indirectly by reducing the phosphorylation of its ligase MDM2. This suggests that MK2 plays a role in regulating p53, as inhibition of MK2 increased the levels of active p53 inside the nucleus. We also found that the combination of TMZ and MK2 inhibition did not induce G\textsubscript{2}/M arrest as expected, but G\textsubscript{0}/G\textsubscript{1} arrest. This increase of cells in G\textsubscript{0}/G\textsubscript{1} led to cellular senescence through the reactivation of the p53-p21 pathway. Moreover, we found that MK2 inhibition enhances the efficacy of TMZ by attenuating long-term clonal and 3D spheroid growth in p53 proficient cells, unlike previously described.

Conclusion
The combination of MK2 inhibition alongside TMZ provides a new therapeutic strategy to enhance the effectiveness of current chemotherapy in p53 proficient tumours.