were analysed by MTS assay and colony formation were assessed by clonogenic assay; Proteins expression were detected by western blotting. Targeted metabolomics were done using LC-MS with stable isotopes labelled intermediates to identify potential metabolic flux changes in GLUL knockdown cells.

**Results and discussions** In vitro analyses of cell viability assay showed that GLUL knockdown in A549 cell line mediates resistance towards the treatment of chemotherapeutic drugs including Pazopanib, Imatinib, Dasatinib and Docetaxel. While, western blotting analyses revealed that reduction in apoptotic marker PARP cleavage in knockdown cells in response to chemotherapeutic drugs with respect to controls. Further, we also observed that GLUL knockdown activates pro-survival signalling mechanism i.e., PI3K-AKT pathway via increased phosphorylation of AKT\text{ser}^{473} and S6 ribosomal protein\text{ser}^{235/236} in mediating resistance. Additionally, in clonogenic assay, cells were grown in the presence of different chemotherapeutic drugs, evidently GLUL knock down cells were able to form more colonies than control cells. Since GLUL activity potentially can be linked to central energy metabolism via glutaminolysis we probed the metabolic fluxes in glycolysis and TCA pathways by means of 13C labelled Glutamine, Glutamate and Glucose respectively and interestingly we didn’t observe any significant changes in metabolic flux linking the resistant phenotype to alterations in energy utilisation by the cells.

**Conclusion** Our study shows that GLUL Knockdown could induce chemo-resistance, through modulating apoptotic and pro-survival signalling. Thus, GLUL may serve as a potential target for NSCLC tumour progression with therapeutic significance.

**PO-250**

**INVESTIGATING BRF2-DEPENDENT RNA POLYMERASE III TRANSCRIPTION Deregulation in cancer**

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**Introduction** Small subsets of RNA Polymerase (Pol) III genes, including the selenocysteine tRNA gene, rely on the presence of the BRF2 transcription factor. Previously, we identified a redox-sensitive switch (Cysteine 361) which, when oxidised, can down-regulate Pol III transcription at all BRF2-dependent promoters (Gouge et al. 2015).

In cancer cells, BRF2 is frequently amplified and/or overexpressed. We hypothesised that activation of BRF2 is required for maintaining high levels of selenocysteine tRNA, as well as selenoproteins, during prolonged oxidative stress. Selenoproteins are required for redox homeostasis and ROS detoxification and we were able to demonstrate that cancer cells with high levels of expression of BRF2 are more resistant to oxidative stress-induced apoptosis (Gouge et al. 2015).

We therefore set out to comprehensively understand the role of BRF2 activation in oncogenesis and its link to oxidative stress response pathways, which are often deregulated in cancer cells.

**Material and methods** HAP1 cells (fully haploid human CML-based cell line) were generated that carried a C361A mutation, which is a redox-insensitive mutant of the molecular pin. These were tested for their proliferative capacity and ability to withstand exogenous oxidative stress. Full length human BRF2 was overexpressed using lentiviral vectors in malignant and non-malignant cell lines; proliferative capacity and BRF2-dependent transcripts were monitored.

**Results and discussions** We found that C361A HAP1 cells were more tolerant to exposure to exogenous oxidative stress using tBHP. However, the proliferative capacity of HAP1 3D spheroids without exogenous stress was not significantly altered by the presence of this mutation. We are investigating the effects of exogenous stress exposure in these cells to identify which BRF2-dependent transcripts are altered following exposure to tBHP.

Overexpression of BRF2 in non-malignant cells is not sufficient to induce increased proliferative potential. Experiments to elucidate the effects of overexpression in malignant cell lines are still ongoing.

**Conclusion** We are continuing to investigate the effects of BRF2 amplification and overexpression on the transcriptional programme of cancer cells under oxidative stress conditions. Since selenocysteine-containing proteins have been implicated in cellular response to ferroptosis, we would like to investigate whether this is mediated by BRF2 overexpression and whether this can be exploited in a therapeutic setting.

**PO-251**

**MODULATION OF NAD+/NADH LEVELS BY NQO1 ENZYMATIC ACTION ALLEVIATES ADRIAMYCIN-INDUCED CARDIAC DYSFUNCTION IN MICE**

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**Introduction** Adriamycin (ADR) is a potent anticancer drug widely used to treat a variety of human neoplasms. Nevertheless, its clinical application is hampered because of severe side effects such as cardiotoxicity and heart failure. ADR-induced cardiomyopathy (AIC) has been reported to be caused by myocardial damage and dysfunction through oxidative stress, DNA damage, and inflammatory responses. But, the remedy for ADR cardiomyopathy is still not developed. We describe the effect of NAD+/NADH modulation by NAD(P)H quinone oxidoreductase 1 (NQO1) enzymatic action on AIC.

**Material and methods** AIC was established by three times intraperitoneal injections of ADR (cumulative dose of 12 mg/kg i.p.; 4 mg/kg every day for 3 consecutive days) in C57BL/6 wild-type (WT) and NQO1 knockout (NQO1\text{−/−}) mice. Before and after exposure to ADR, the mice were orally administered dunnione (20 mg/kg), a substrate of NQO1. Cardiac biomarker levels in the plasma, cardiac dysfunction, oxidative biomarkers, and mRNA and protein levels of pro-inflammatory mediators were determined to compare the cardiac toxicity of each experimental group.

**Results and discussions** All biomarkers of cardiac damage and oxidative stress, and mRNA levels of pro-inflammatory cytokines, including cardiac dysfunction were significantly increased in ADR-treated both WT and NQO1\text{−/−} mice. However, this increase was significantly reduced by dunnione in WT, but not in NQO1\text{−/−} mice. In addition, a decrease in SIRT1 activity due to a decrease in the NAD+/NADH ratio by PARP-1 hyperactivation was associated with ADR-induced cardiotoxicity through increased nuclear factor (NF)-κB p65 and p53 acetylation in both WT and NQO1\text{−/−} mice, whereas an increase in NAD+/NADH ratio by NQO1 enzymatic action using dunnione as a substrate recovered SIRT1 activity and