**Introduction**

- Lung cancer is the largest cancer killer with poor 5-year survival rate. Non-small cell lung cancer (NSCLC) patients undergo primary, adjuvant or neoadjuvant radiotherapy treatment for NSCLC with image-guided radiotherapy (IGRT) being widely used to treat cancer patients providing benefit with more accurate treatment plans and reduced side effects.

- NSCLC patients that have activating mutations in the EGFR gene are treated with epidermal growth factor receptor (EGFR) inhibitors e.g. Erlotinib (Tarceva) and Gefitinib (Iressa). However, resistance emerges in the majority of patients due to secondary gatekeeper mutations (T790M). CR is minimal amplification of genes such as c-MET and Her2.

The HCC827 NSCLC adenocarcinoma cell line, which harbours an activating EGFR mutation (del E746-A750), was used to generate EGFR inhibitor (EGFRi) resistant models.

Here we demonstrate the application of the image-guided small animal radiation research platform (SARRP, Xstrahl Ltd) to treat subcutaneous xenograft tumours with irradiation, using planned protocols similar to those utilised in the clinic, with little or no adverse effects on mice and to report on combination treatment strategies to overcome EGFR inhibitor resistance.

**Methods**

- **Generation of resistance variants of HCC827**; EGFR resistant variants of HCC827 (ER1 and GR1) were generated in vitro following subculture with escalating doses of Erlotinib or Gefitinib respectively. Further models of HCC827 were generated consisting of single-cell cloning of the resistant HCC827 variants. HCC827 and the resistant variants were characterised for c-MET genomic amplification by qPCR (RNAseq reference) and Axin gene expression via RT-PCR (HPRT reference). STR profiling was also carried out (LG Promochrom). Resistance was also generated in vivo by allowing tumours to outgrow under dosing pressure. Xenograft tissue was excised, disaggregated and purified into cells; this cell line is designated PCS030.

- **In vitro IR assay**; Cells were grown in T25 flasks and treated with irradiation and counted after 6 days. For IC50 evaluation, cell lines were seeded in 24-well plates and viability assessed by CellTiter Blue (Promega).

- **In vivo studies**; Cells were implanted subcutaneously in nude mice (Vivatools; in HsdOla:MF1); i.e. HCC827 was dosed at 25mg/kg po ID and Crizotinib was dosed at 50mg/kg po OD. Tumour measurements and body weights were taken 3 times weekly and dosing initiated in the 2 models when the tumours reached a mean volume of ~200mm3.

- **In vivo Irradiation**; Mice were anaesthetised and transported to the SARRP where CRCT images were acquired. Using the BioSoftware the isocentre of the tumour was identified and aligned with the central axis of the gantry. Fractionated irradiation was administered with the SARRP (225 kV peak x-ray beams, dose rate of 2.5 Gy/min) using collimators of various dimensions and a double beam (gantry position at 0° and 180°) under the guidance of the CRCT. A tolerability was performed initially to evaluate 30Gy/day x 5 days for 2 weeks.

**Results: Generation of resistance**

- EGFR resistant variants of HCC827 (ER1 and GR1) exhibited a >500 fold shift in IC50 (Figure 1). Single cell colonies were generated and characterised for c-MET genomic amplification by qPCR.

**Results: In vitro sensitivity to irradiation**

- In vitro for life...