**Background**

Most non-small cell lung cancer (NSCLC) patients that have activating mutations in the EGFR gene will respond to treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) e.g. Erlotinib (Tarceva®) and Gefitinib (Iressa®).

However, following months of dosing, in about 50% of these cases a secondary mutation in EGFR (T790M) will lead to treatment failure, suggesting that other mechanisms of clinical resistance can also occur. Other mechanisms of clinical resistance can also occur in these cases a secondary mutation in EGFR (T790M) 

EGFR-TKI resistant variants were generated and characterised for their resistance mechanisms (programme on-going).

EGFR-TKI resistant variants were generated in vitro from the HCC827 NSCLC cell line and resistance to EGFR TKIs translates to the in vivo setting.

**Methods**

HCC827 is an NSCLC adenocarcinoma cell line with an activating EGFR mutation (del E746-A750). The cells were used to confirm the generation of EGFRi resistant variants of HCC827 (ER1 and GR1) were assessed in the parental line and 1:1 and 100:1 (Erlotinib/Crizotinib) in the parental line and 1:1:1 and 100:100:1 were tested in resistant.

Combination treatment (c-Meti and EGFR-TKI) overcomes in vitro EGFR-TKI resistance.

**In vivo generation of EGFRi resistance (PDX)**

Mice were dosed with Erlotinib or Gefitinib (125 or 25mg/kg) o.d. in treatment cycles of up to 10 weeks followed by outgrowth and re-passage. Graphs show the initial treatment-naive growth profile in two example mice (data expressed as % of the initial dose volume) overlaid with the growth profile following 3 cycles of dosing pressure (equivalent to up to 4 months of q.d. dosing).

**LU6422 response to Erlotinib in vitro**

Dosing was initiated on day 27 (arrowed) and partial regression of all tumours was observed at day 60 (90mg/kg and 25mg/kg Erlotinib p.o. q.d.) error bars represent SEM. Caspase Dylight (Thermo Scientific) gives an early read-out (50th post-treatment p<0.05) of efficacy in vivo via Spectrum CT (PerkinElmer Corp).

**Generation of EGFR-TKI resistant in HCC827 in vitro**

EGFR-TKI resistant variants of HCC827 (ER1 and GR1) were generated in vitro following sub-culture with escalating doses of Erlotinib or Gefitinib respectively.

**Combination index in vitro**

Synergistic effect was calculated based on combination index values according to the Chou and Talalay method (CompuSyn). Combination index values were determined in a dose–response setting.

**Summary**

EGFR TKI resistant subtypes were generated in vivo from a proprietary Caucasian NSCLC PDX model (LU6422) and characterised for their resistance mechanisms (programme on-going).

EGFR-TKI resistant variants were generated in vitro from the HCC827 NSCLC cell line and resistance to EGFR TKIs translates to the in vivo setting.

Cross-resistance to EGFR TKIs was observed in resistant HCC827 variants along with an elevation in the c-MET gene copy number which correlated with combination efficacy in vitro.

Combination treatment (c-Meti and EGFR-TKI) overcomes in vivo resistance in c-Met driven EGFR TKI resistance.

**Conclusions**

Combination treatment as described above are critical to the successful treatment of various NSCLC variants which seek to prevent or overcome the emergence of resistance to EGFR TKIs.

Offer proof of concept for generation other resistant lines/models for current or new treatment strategies for MAb or small molecules.

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