**Novel RET+ patient-derived cell lines reveal unique signaling dynamics and dependencies**

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### INTRODUCTION

- RET-gene fusions occur in approximately 1%-2% of NSCLC and also occur in CRC, thyroid and other cancers.
- RET fusion results in the expression of a ligand-independent, constitutively active fusion kinase.
- RET fusion proteins activate downstream pathways, including MAPK and P38, to promote growth and proliferation.
- In lung cancer, approximately 70% of RET fusion partners are KIF5B, however, several different RET fusions have been identified including CCDC6, TRIM33, RCCA, TRIM6, and others.
- Multikinase inhibitors (including but not limited to venetoclax, cabozantinib, ponatinib, sorafenib, sunitinib) with potent activity against RET have variable response rates in RET+ patients.
- Novel, selective RET inhibitors are currently being tested in clinical trials.
- Phase I/II trials using the selective RET inhibitor BLU-2600 have shown efficacy in preclinical models of RET+ patients.
- To date, there have been a limited number of studies to evaluate RET signaling and regulation in the context of NSCLC.

### RESULTS

**RE+ cells exhibit differential sensitivity to RET and MEK inhibitors**

**Inhibition of Src sensitizes CUTO32 cells to RET inhibitors**

**Drug screen reveals CUTO32 cells are uniquely dependent on GSK cell-cycle regulation**

### CONCLUSIONS

- We have derived three novel RET+ cell lines, CUTO32, CUTO32 and CUTO42, which demonstrate differential sensitivity to RET inhibitors ponatinib and ROX-105.
- KIF5B-RET is successfully inhibited with other Ponatinib or ROX-105.
- Differential signaling through RET in AKT is more robustly inhibited then ERK in LC24/4A and CUTO42 cells. Additional CUTO32s are resistant to sensitivity, suggesting they may be MAPK pathway-independent.
- SRC contributes to RET inhibitor resistance in CUTO32 cells.
- The CUTO2 cell line is uniquely sensitive to PK1 and Aurora A kinase inhibitors, compared to the CUTO2 cell line. The CUTO32 and CUTO42 cells displayed intermediate sensitivity.
- GSK3α/δ reveals that CUTO32 cells have an advantage for the CUTO32 cell line over the other CUTO32 cells.
- Overall, these RET+ cell lines reveals that RET neomutations can differently regulate downstream pathways and different sensitivities to kinase inhibitors.