OXFORD UNIVERSITY INNOVATION

Non-confidential information

IGF2-TRAP (15455/15456)

Not for further distribution

IGF2-TRAP Background

- The introduction of five mutations (AB5) decreased the KD from 247nM (WT) to 5.1nM, an improvement of 48-fold.

- Three further mutants were selected: AB5-Q1569R (AB+CD loops), AB5-P1597H-S1602H (AB+FG loops) and AB5-Q1569R-P1597H-S1602H (AB+CD+FG loops; since referred to as domain11$^{AB5\text{-}RHH}$), with the latter triple mutant having a drop in KD to 0.65nM (412-fold increase over WT).

In vivo activity of ligand trap

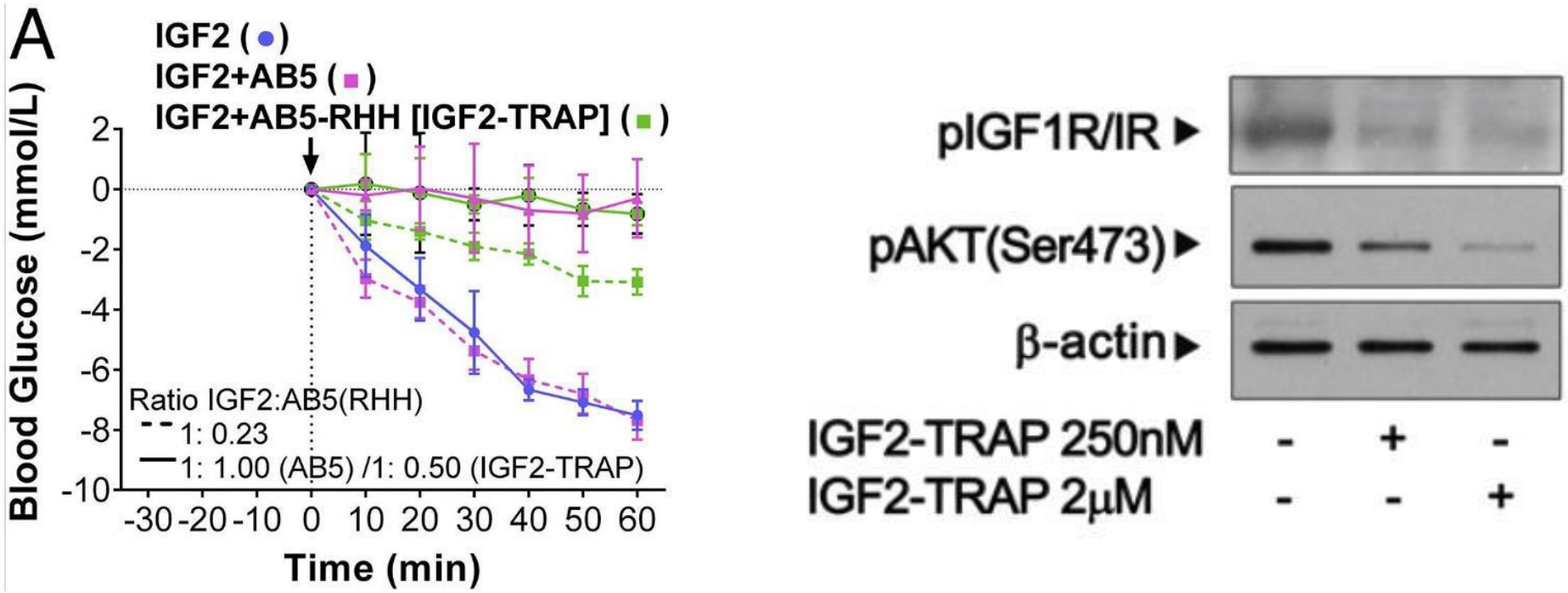

Figure 1.FC domain 11^{AB5} and domain$11^{AB5\text{-}RHH}$ ligand trap inhibit IGF2 signalling *in vivo*. Both ligands abrogate $IGF2^{1\text{-}67}$ induced hypoglycaemia in a mouse model(a), at various molar ratios. This effect is attributable to reduction in downstream activation of AKT signalling, as measured by a reduction in phosphorylation (b).

Effect on xenograft growth and serum levels

An autocrine-IGF2$^{1-67}$ dependent tumour model was developed using the Ewing sarcoma cell line (SKNMC). Retroviral-transformed SKNMC cells were injected into adult mice (which do not express IGF2), 24 hours after IgG2-Fc Domain11$^{AB5-RHH}$ was infused into the mice using an osmotic minipump. A modest effect on tumour volume was observed (*Figure 2a*) and circulating IGF2$^{1-67}$ was less evident in treated mice, without any alteration in either total serum IGF1, GH or IGFBP levels (*Figure 2b*).

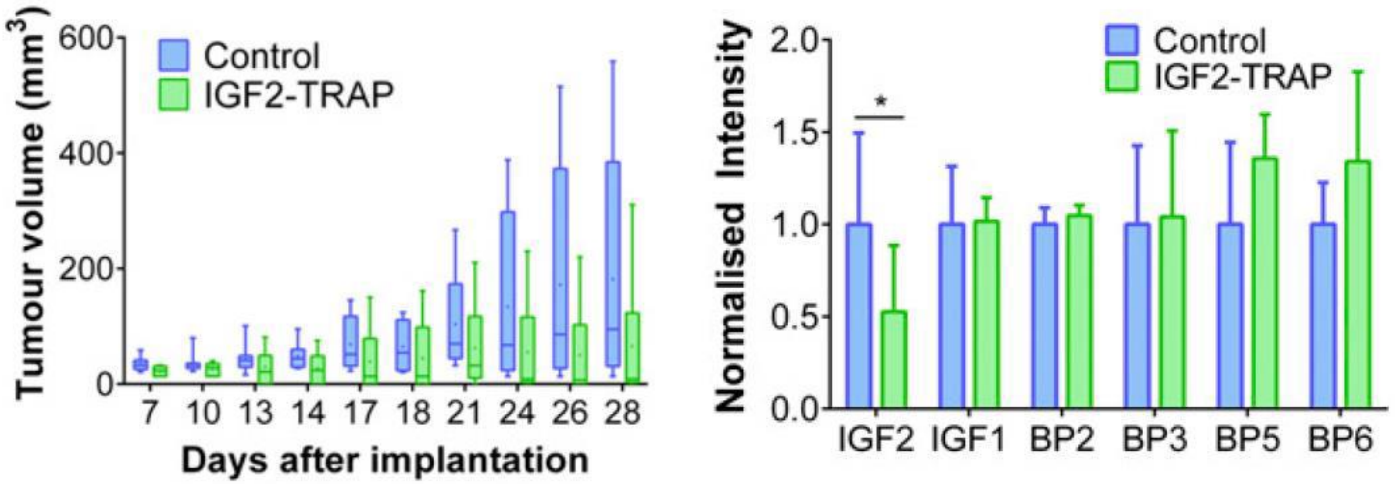

Figure 2. FC domain11$^{AB5-RHH}$ reduces IGF2-dependent xenograft growth (a) and reduces levels of serum IGF2, independent of IGF1, GH and IGFBP level.

Identifying synergistic compounds

233 compounds selected from an oncology library and 77 therapeutics from the DTP Approved Oncology Drug Set were screened for a reduction in cell viability, in combination with the ligand trap Two PI3 kinase inhibitors (PF-04691502 and Pictilisib) demonstrated synergistic activity

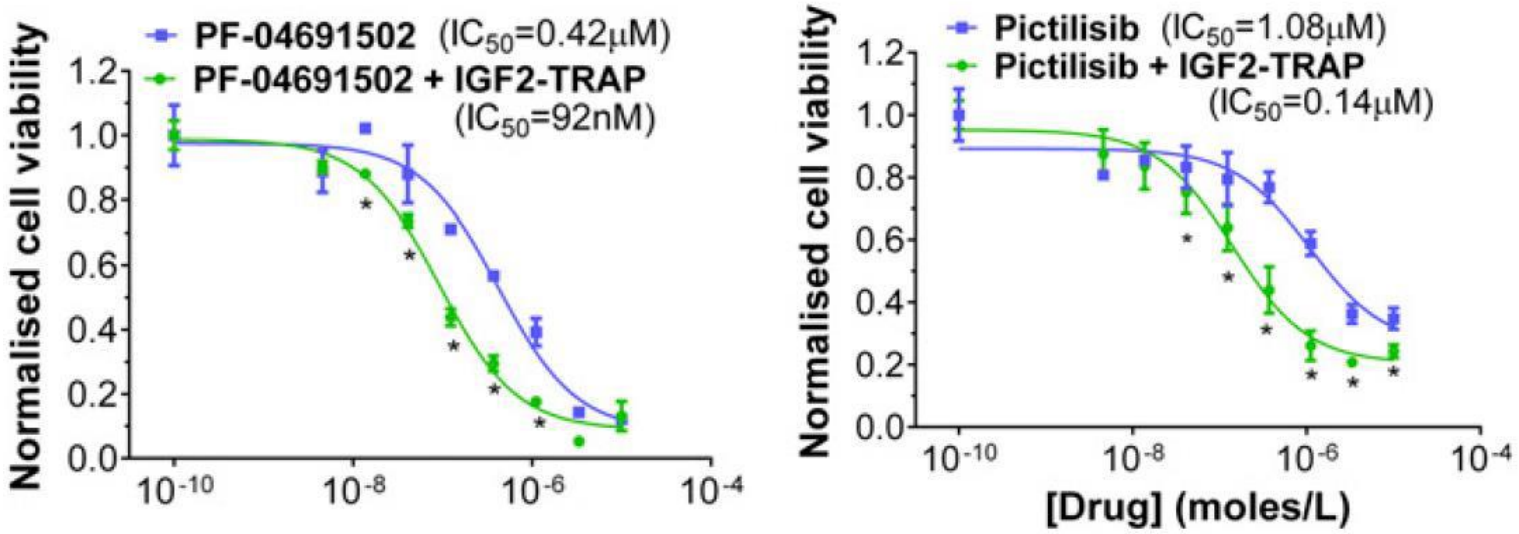

Figure 3. Validation dose-response curves for PI3 kinase inhibitors (PF-04691502 and Pictilisib) in the presence (green) or absence (blue) of IGF2-TRAP.IC50 values are shown.

Neutral size-fractionation of serum from a patient with non-islet cell tumour-associated hypoglycaemia (NICTH) showed that IGF2-TRAP depleted both lower and higher molecular weight isoforms of IGF2, suggesting that the ligand can target big-IGF2 associated with human cancer and NICTH.

IGF2-TRAP *in vivo* stability

- Tested thermal stability (Thermofluor in vitro assay) of various Domain11 mutants. All show an improvement in thermal stability to 61.7-69.1°C

- In vitro analysis of proteolytic stability of IGF2-TRAP, tested with elastase and cathepsin. Both $FcA^{B3\text{-}P/H}$ and Fc^{WT} show reasonable resistance to proteases.

- Attempts to run half-life studies in mouse (1 hour, 72 hours and 1 week) have failed as samples were not amenable to Western Blot quantification. This is to be run instead using labelled IGF2-TRAP by ELISA. Biotinylation of the ligand has been successful, and experiments are currently ongoing.

Further ongoing work:

- Functional cytostatic activity in human cancer cell xenografts
  - Cell lines growing well and dependency on IGF2 confirmed
- Functional cytostatic or cytotoxic activity in human cancer cell mouse xenografts in combination with PI3kinase inhibitors
  - Preliminary work suggests synergistic activity in tested two cell lines

IP

- An patent application was filed in 2005 covering the original set of mutants. This was granted in Europe (granted 2006, EP1945663) and USA (granted 2008, US8293875).

- To extend the patent life, a second patent application was filed internationally in 2015 (WO2017029148A1). The novelty was due to the unexpected improvements in binding brought about by the proline mutation. This second patent is currently being pursued in USA and Europe.