Discovery of a potent KRAS macromolecule degrader specifically targeting tumours with mutant KRAS

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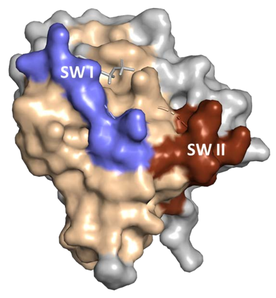
Tumour-associated KRAS mutations are the most prevalent in the three RAS-family isoforms and involve many different amino-acids. Therefore, molecules able to interfere with mutant KRAS protein are potentially important for wide-ranging tumour therapy. We describe here (1) the selection and characterisation of a KRAS-specific antibody mimetic (Designed Ankyrin Repeat Protein, DARPin K19) and (2) the engineering of this DARPin into RAS degraders based on protein macromolecules fused to specific E3 ligases. The KRAS-specific DARPin K19 fused to the VHL E3 ligase is compared to a pan-RAS intracellular single domain antibody (iDAb RAS) fused to the UBOX domain of the CHIP E3 ligase. We demonstrate that while the KRAS-specific DARPin degrader induces specific proteolysis of both mutant and wild type KRAS, it only inhibits proliferation of cancer cells expressing mutant KRAS *in vitro* and *in vivo*. Pan-RAS protein degradation, however, impairs proliferation irrespective of the RAS mutation. These data show that specific KRAS degradation is an important therapeutic strategy to affect tumours expressing any of the range of KRAS mutations.

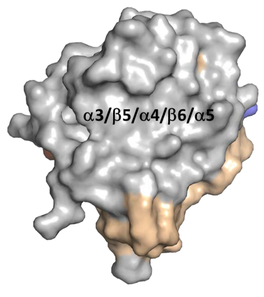
- 100% conserved between K/N/HRAS isoforms
- GDP/GTP selectivity
- Inhibitors binding +++

KRAS protein – effector lobe view

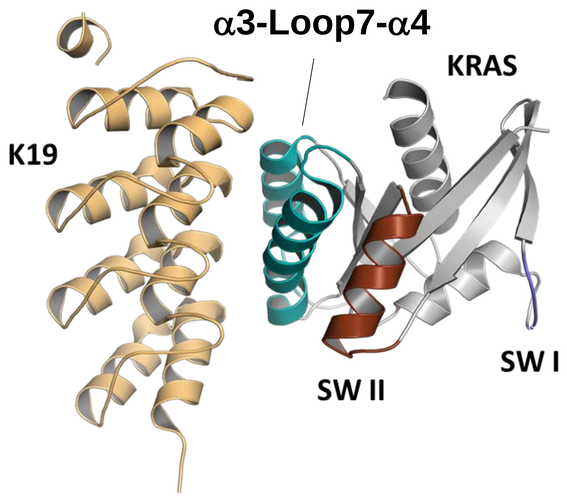


- 90% conserved
- Isoform specificity?

KRAS protein – allosteric lobe view



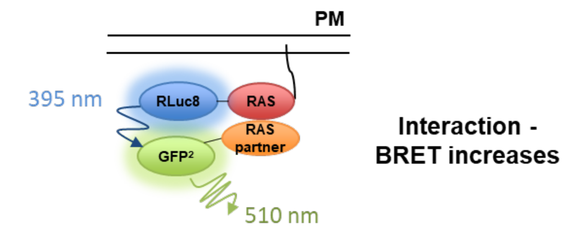
1) K19 binds KRAS allosteric lobe



$KRAS^{G12V}$ / DARPin K19 (6H47)

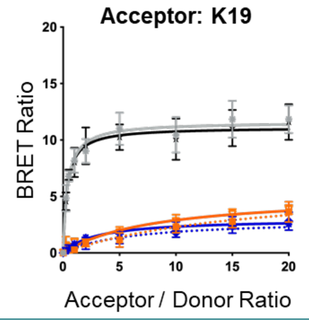
$K_d \approx 10nM$ for KRAS (-GDP and -GTP bound)

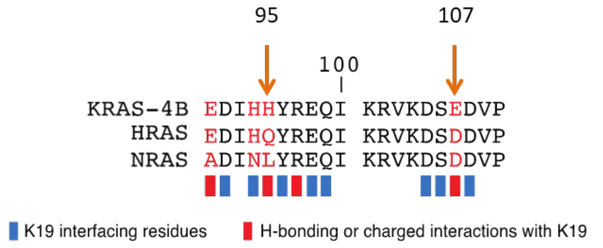
2) K19 binds specifically KRAS through its interaction with H95



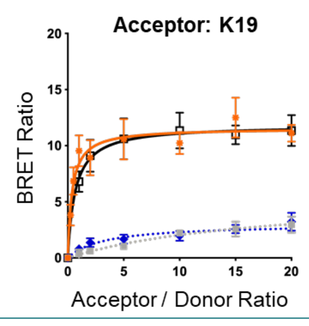
Interaction - BRET increases

Donor: $KRAS^{G12D}$ $KRAS^{WT}$ $NRAS^{Q61H}$ $NRAS^{WT}$ $HRAS^{G12V}$ $HRAS^{WT}$



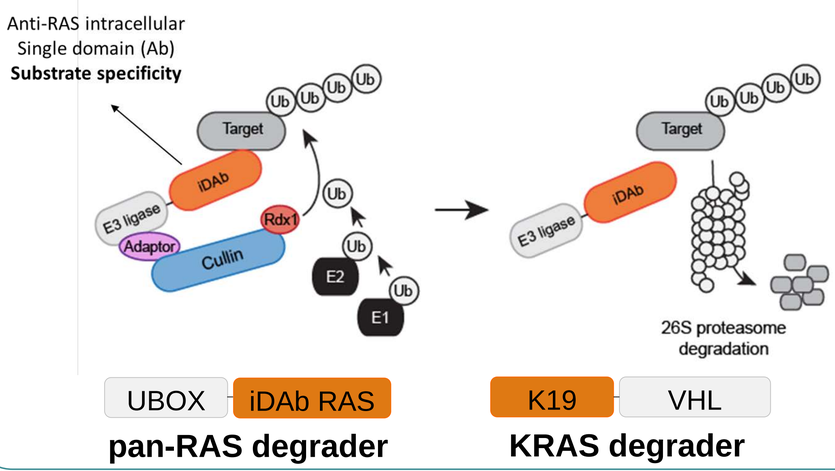


Donor KRAS: WT, E107D, H95Q, H95L

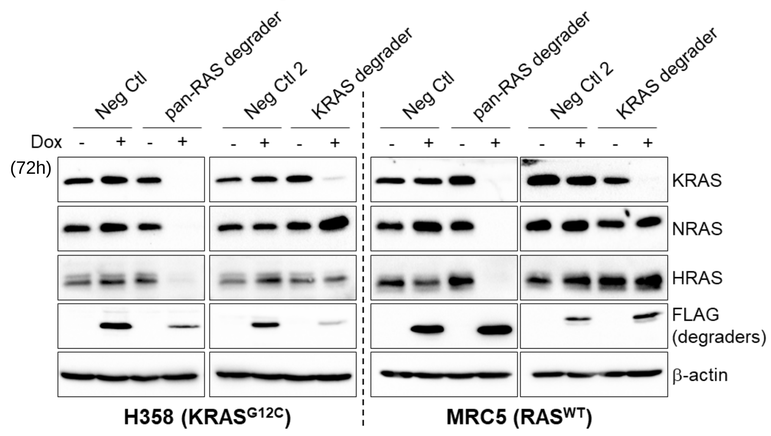


3) Development and characterisation of pan-RAS and KRAS-specific macromolecule degraders

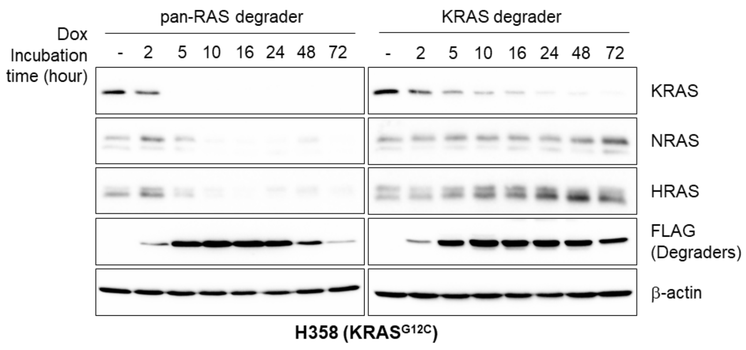
a) Single domain-based targeted-protein degradation



b) Efficient and specific depletion of endogenous RAS in cells

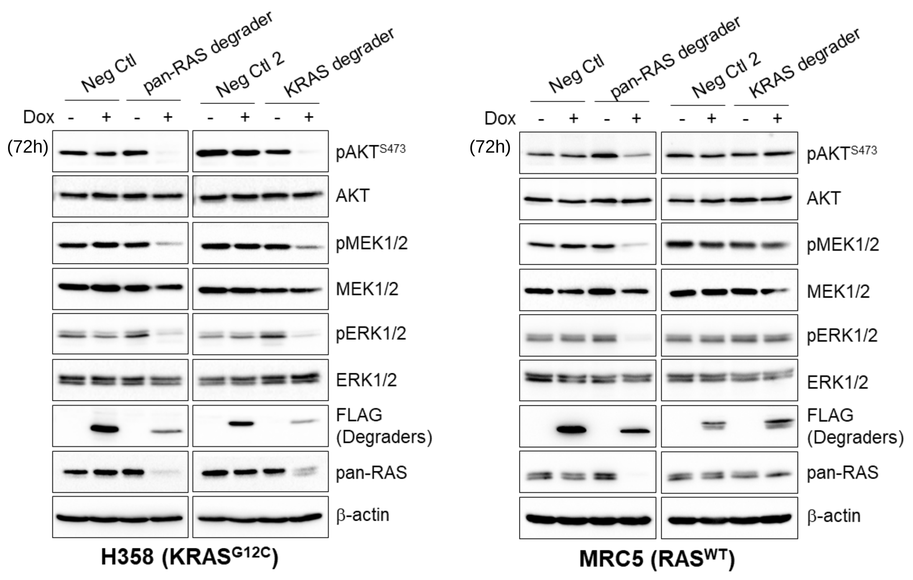


c) Fast, sustained and specific degradation

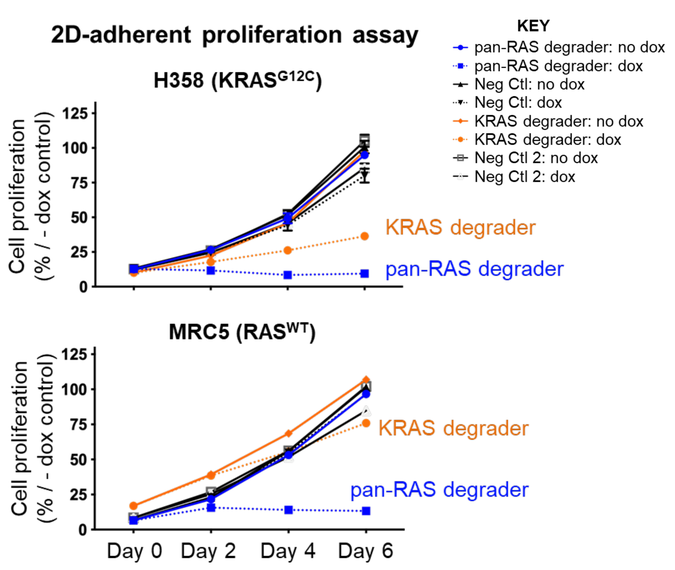


4) Specific inhibition of mutant KRAS cancer cells and tumours with KRAS degrader

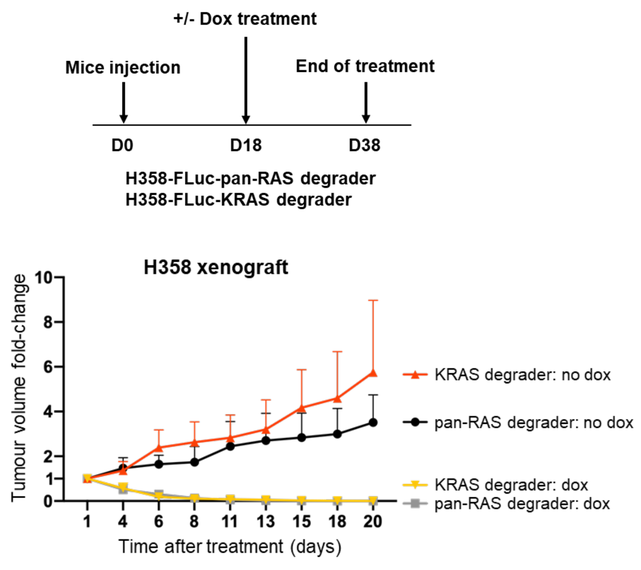
a) KRAS degrader impairs RAS signalling specifically in mutant KRAS cancer cells

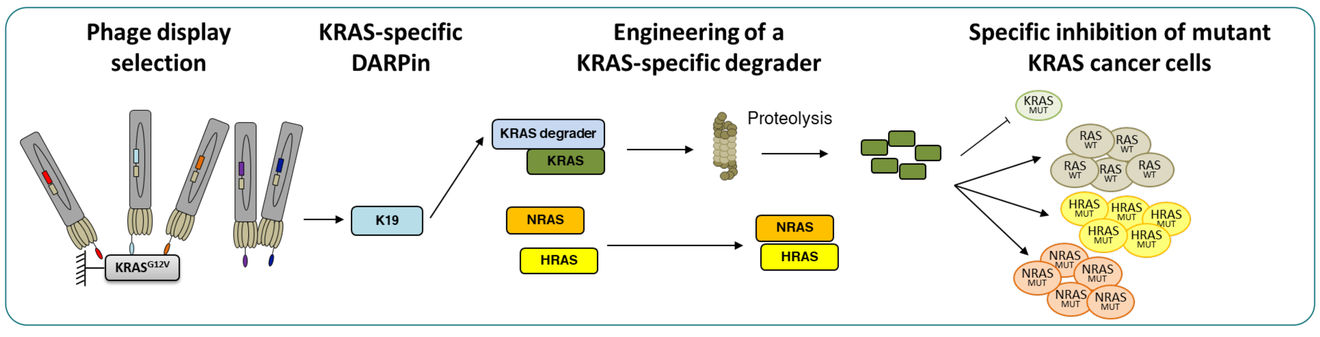


b) KRAS degrader inhibits specifically mutant KRAS cancer cells growth *in vitro*



c) KRAS degrader induces regression of mutant KRAS driven tumours *in vivo*





This work was published in:
Bery N, et al. Nature Communications. 2019 (KRAS-specific DARPins)
Bery N, et al. Nature Communications. 2020 (macromolecule-based RAS/KRAS degraders)

The schematic diagram displayed in 3) was adapted from Bery N, et al. Cell Chem. Biol. 2019

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