Screening for RAS inhibitor combination targets using a multiplexed CRISPR platform that incorporates drug sensitivity profiling, mode of action assays and cell line generation

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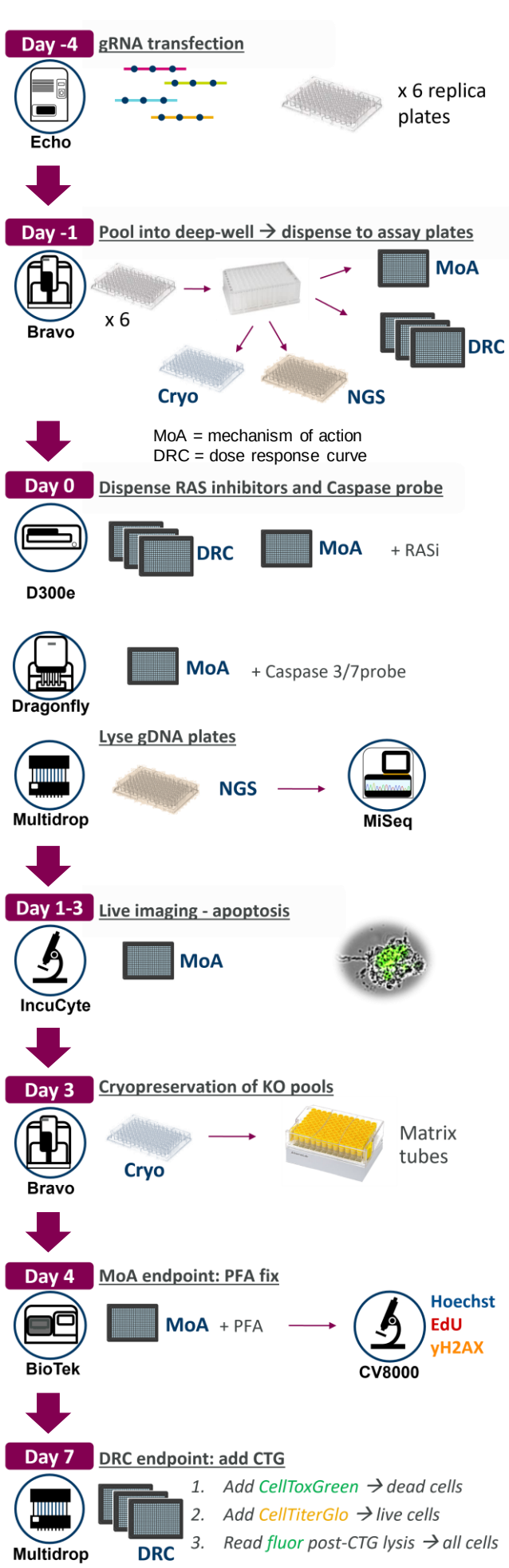
Background

The challenge: Requirement for rapid target validation (TV), following a whole-genome pooled screen which identified sensitisers to RAS inhibition (RASi) in pancreatic (PDAC) and colorectal (CRC) cancer cell lines.

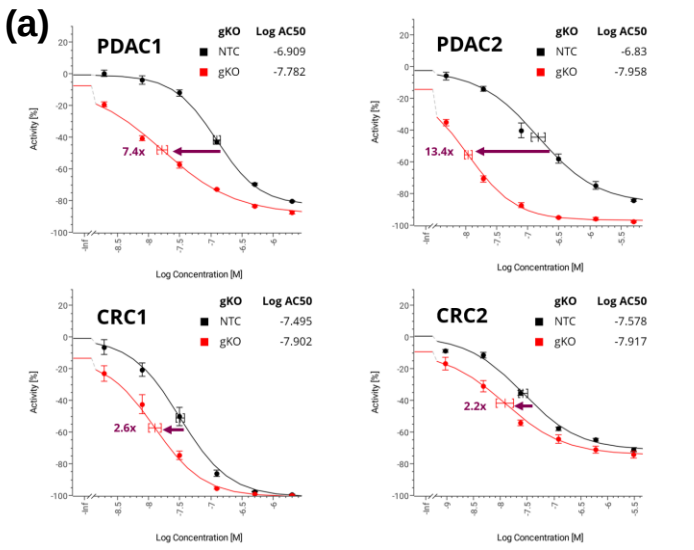
The aim: Develop a semi-automated TV workflow that parallelises drug sensitivity profiling, mode of action assays and cell line generation.

Methods

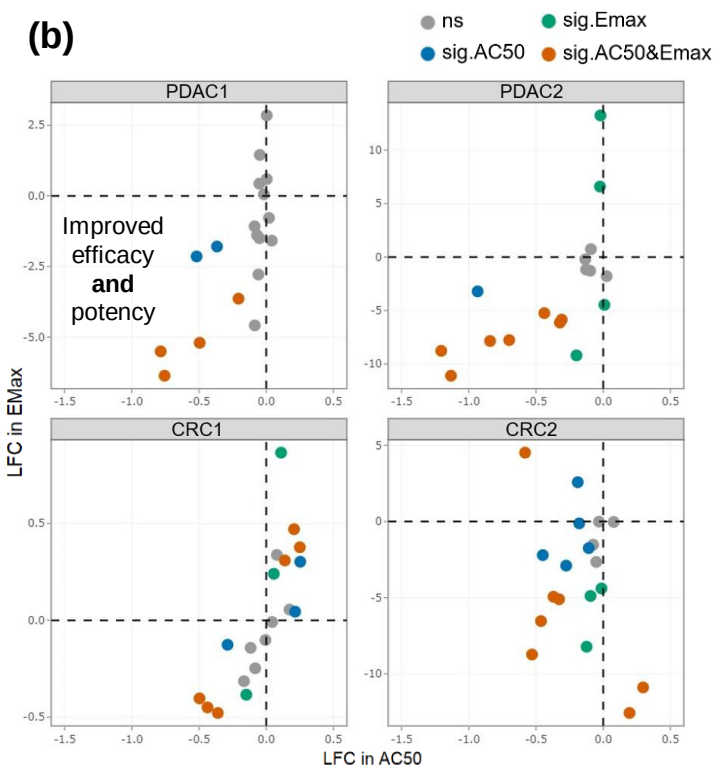


Results

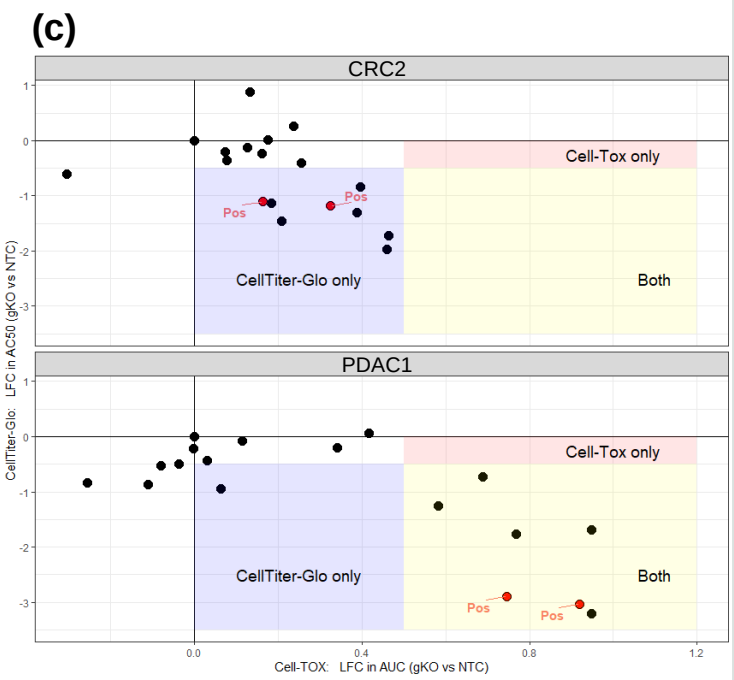
Drug sensitivity profiling



(a) RASi dose response curves from pilot screen CellTiter-Glo assay, in four cell lines. Red = positive control gKO. Black = neutral control (NTC).

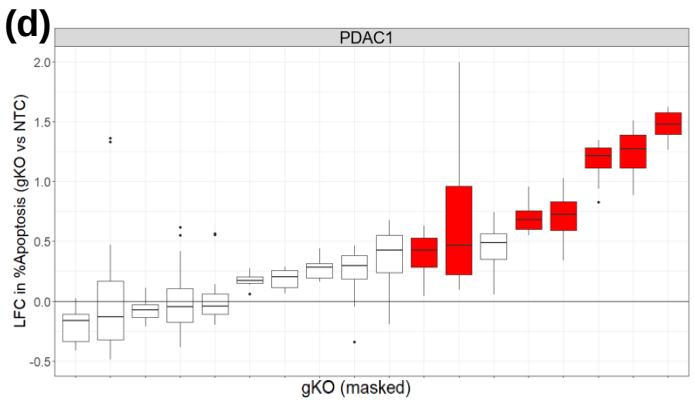


(b) Pilot screen hit calling, based on changes in RASi potency (AC50) and efficacy (Emax). Bottom-left quadrant = improved efficacy *and* potency. LFC = log10- fold change.



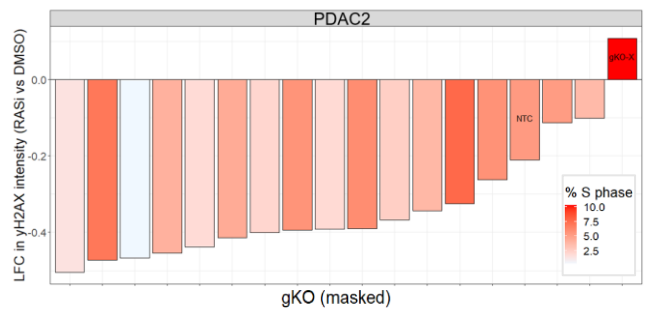
(c) Cytotoxic vs cytostatic: in PDAC1, most hits sensitised in both DRC assays (CellTiter-Glo and CellTox). For CRC2 hits, RASi remained cytostatic. Red = positive control gKO. LFC = log2-fold change.

MoA assays – apoptosis



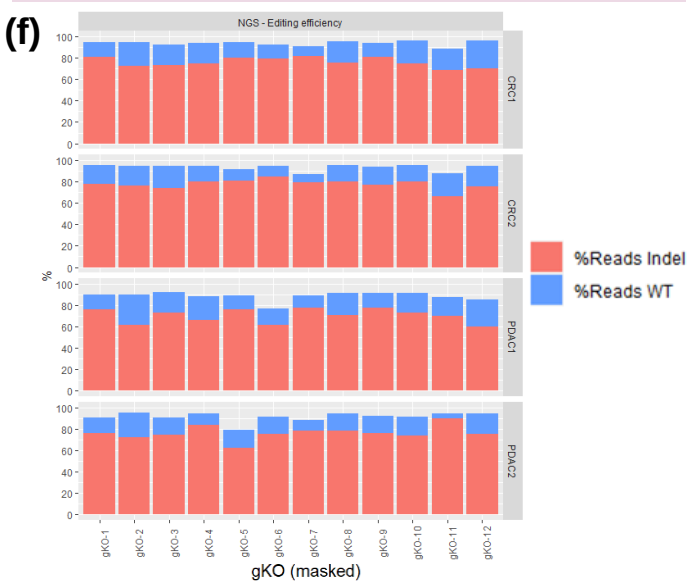
(d) %Apoptosis. Red = cytotoxic effect seen in CellTox DRC assay (fig c). LFC = log2-fold change.

MoA assays – DNA damage & cell cycle



(e) In NTC cells, and most gKOs, RASi reduced γH2AX intensity compared to DMSO. In gKO-X, we observed an increase in γH2AX and a high % S phase fraction. LFC = log2-fold change.

Cell line generation & NGS



(f) NGS on every gKO revealed high indel efficiencies (mean >75%). All KO pools were frozen, to enable rapid follow-up studies.

Conclusions

- Using a multiplexed workflow, we have run a pilot TV screen that successfully identified genes which sensitise to RASi, increase apoptotic cell death, modulate the DNA damage response and disrupt the cell cycle.
- By running the drug sensitivity profiling in conjunction with the MoA assays, plus the cell line generation, we estimate that we have sped up the hit annotation process by 2-3 months.
- Sensitisers identified using this screening setup could be novel combination targets with RASi.