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RNA-based screens in primary human immune cells

Bronwyn Joubert, Cristina Ghirelli, Isabelle Nett, John Prime, Glynn Martin, Carlos le Sage, Jonathan Moore, Benedict Cross and Nicola McCarthy

Horizon Discovery Ltd, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, UK

Introduction

Pooled CRISPR knockout screens in primary human T cells allow clinically relevant biological questions to be answered in a cellular context that is more relevant to the clinic. Any potential new targets or biological behaviours identified in these screens could translate more robustly to the clinic, saving time and money.

- As a proof of concept study for a pooled CRISPR knockout screen in ex vivo human T cells (Figure 1), we chose to use a metabolic library to look at a drug-gene interaction paradigm similar to that previously published by Birsoy *et al.*, (2015).
- 4,815 short guide RNAs (sgRNAs) targeting 482 genes involved in metabolism were cloned into our lentiviral backbone and the metabolic and control libraries (2442 guides targeting 152 genes and 100 non-targeting guides) were mixed at equimolar ratios and lentivirus generated.
- Fresh $CD3^+$ T cells were isolated from three healthy donors, stimulated with anti-CD3 and anti-CD28 antibodies and transduced with the pooled sgRNA library.
- After puromycin selection, T cells successfully transduced with sgRNAs were electroporated to transiently introduce Cas9 mRNA.
- The CRISPR-Cas9 edited pool of cells was exposed for seventeen days to a dose of phenformin that results in growth inhibition and not cell death (Birsoy *et al.*, 2015).
- T cells were harvested at several time points during the drug treatment, and genomic DNA was extracted.
- NGS was used to quantify the relative abundance of sgRNAs in each sample. Each sample was normalised to the plasmid library, and a comparative analysis conducted using either an RRA-based approach (Li *et al.*, 2014) or a Z-score based approach (Wang *et al.*, 2017).

Schematic of T cell screen

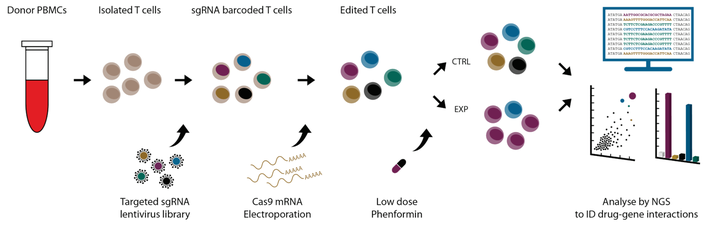


Figure 1 | T cell response to drug

A 10 µM dose of phenformin was chosen to drive the selective pressure in the screen because this dose inhibits the growth of the primary T cells without killing them. This concentration was different to that published for Jurkat cells (Birsoy *et al.*, 2015). Despite T cell donor variability the response to treatment was robust and suitable for hit identification.

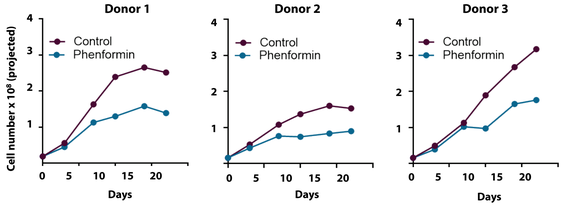
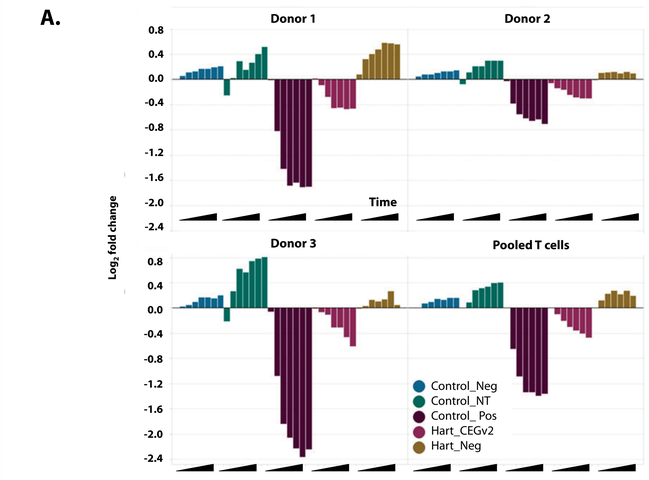
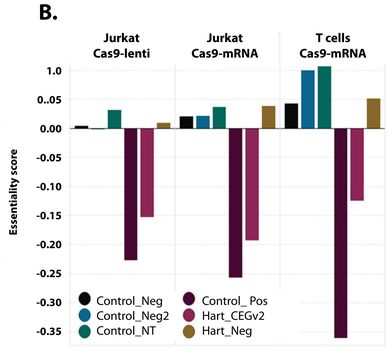


Figure 2 | Analysis of library performance

To assess the performance of our library, we evaluated the dynamics of control gene behaviour on a per donor basis and in the primary T cells as a whole compared with Jurkat T cells. We used our published control genes (Cross *et al.*, 2016) and those described by Hart *et al.*, (2016).

A. Essential genes dropped out as expected over time. This was clearly visible in all 3 T cell donors, but there was some variation in the log2 fold change between the T cells isolated from different donors. Negative control genes [Control_Neg and Hart_Neg Hart *et al.,* (2016)]; non-targeting guides [Control_NT]; and core essential genes [Control_Pos and Hart_CEGv2 Hart *et al.,* (2016)].
B. Control gene performance was better in the primary T cells compared with Jurkat cells screened using our combined lentivirus and electroporation approach or with historic Jurkat CRISPR screen data using our all-in-one vector to deliver both guides and Cas9 simultaneously.
C. Pooling of the data from the T cells isolated from different donors improved the statistical capacity to detect true positive essential genes (ROC curves).





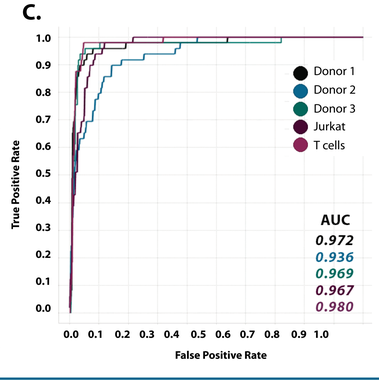
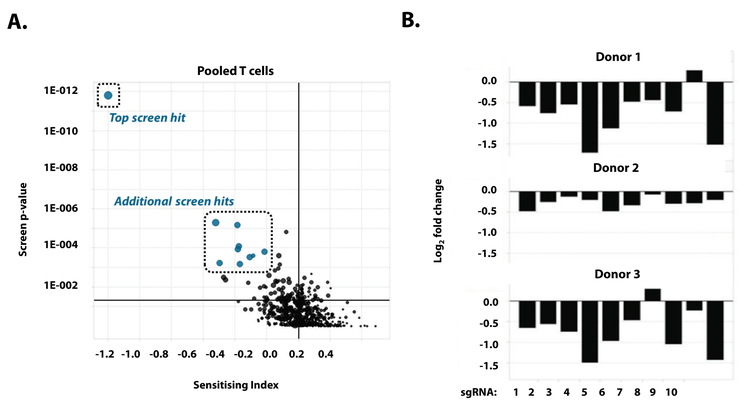
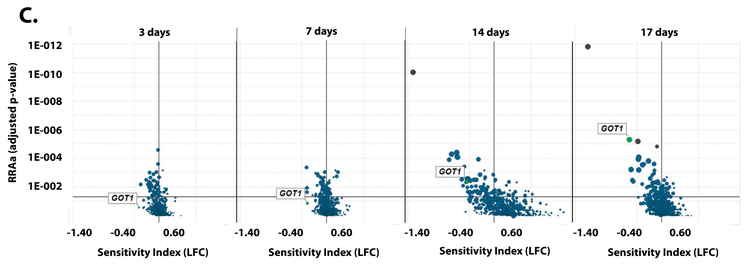


Figure 3 | Drug-gene interaction analysis and hit calling

Evaluation of the data from the drug-gene interaction screen identified genes that were lost from the phenformin-treated cells compared with control cells (sensitivity analysis).

A. *GOT1* loss is a key sensitising factor in primary T cells in agreement with the Jurkat T cell data published by Birsoy *et al.*, (2015). Moreover, our analysis identified additional genes, which on deletion increased phenformin cytotoxicity.
B. The library guides targeting *GOT1* perform consistently with good reproducibility across replicates. Donor variation manifests mostly as a dampened guide drop out in donor 2 but removal of donor 2 does not substantively alter hits .
C. The time-dependent effects of drug-gene hit identification was explored and there is robust hit identification at the final time point, indicating the importance of a long assay window to robustly identify hits.





Conclusions

- This proof of concept study shows that pooled CRISPR-Cas9 screening in primary T cells is possible using a combined lentivirus and electroporation approach.
- These assays provide a useful method of identifying novel potential hits, which when validated, could provide new therapeutic targets.
- We are currently developing new CRISPR-Cas9 arrayed screens in primary immune cells, such as myeloid-derived suppressor cells, monocyte-derived dendritic cells, natural killer cells and B cells.

References

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