Pooled and arrayed CRISPR-Cas9 knockout screens in primary human immune cells

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CRISPR knockout screens in primary human T cells can help to address clinically relevant biological questions *in vitro*. Any potential new target or biological behaviour identified in these screens could translate more effectively from bench to bedside, saving time and money. Pooled and arrayed CRISPR screens address different questions: Pooled screens are effective for analyzing impacts of gene loss at a population level, whereas arrayed screens allow analyzes of individual genes on a well-by-well basis and are more applicable to complex phenotypic endpoints. Our proof-of-concept studies demonstrate the utility of pooled and arrayed CRISPR knockout screens in primary human immune cells.

Using a combined lentivirus and electroporation approach developed in house, we analyzed drug-gene interactions evident in a pooled CRISPR knockout screen in *ex vivo* human CD3+ T cells exposed to phenformin, an electron chain inhibitor. A short single guide RNA (sgRNA) library of over 7,000 sgRNAs was used to target 482 metabolic genes and 152 control genes. Our resulting dataset passed all NGS and screening standard QC on a donor per donor basis and confirmed loss of the gene *GOT1* as a key sensitising factor to phenformin in primary T cells. This finding is in agreement with Jurkat T cell data published by Birsoy *et al*., (2015), and we also identified additional genes which on deletion increased phenformin cytotoxicity.

Although pooled CRISPR screens are useful for analyzing the impact of gene loss in a population of cells, sometimes well-by-well readouts are needed to address more complex biological endpoints, such as co-culture endpoints. We have used a semi-automated electroporation approach to carry out arrayed CRISPR knockout screens in human T lymphocytes and human myeloid cells. For CD4+ T cells, monocyte derived dendritic cells and monocyte-derived macrophages, we have identified electroporation conditions that enable a high percentage of cells in every well to remain viable after successful gene knockout, verified using molecular- and protein-based approaches. We have also carried out functional assays to look at the impact of gene loss. In CD4+ T cells we assessed the impact of CD3ε knock out by co-culturing CRISPR-Cas9 edited T cells with unedited monocyte-derived dendritic cells. Multiplexed HTRF and FACS analyses showed that CD3ε-edited CD4+ T cells had reduced proliferation, decreased IFN-γ secretion and lower CD25 expression compared to cells treated with non-targeting guides, as expected.

In summary, pooled and arrayed CRISPR-Cas9 screens in primary T cells will provide useful methods for identifying novel potential hits, which could translate into therapeutic targets. Pooled CRISPR screens are applicable to primary immune cells that can be produced in large numbers, whereas arrayed CRISPR screens can be used to assess the impact of gene knockout in immune cells that are less abundant and to assess physiological, co-culture endpoints that are not suited to a pooled population approach.

Birsoy, K. *et al.* (2015) An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. *Cell* **162**, 540.