Arrayed CRISPR screening in primary human T_{regs} using a multi-colour flow cytometry endpoint: automation yields high KO efficiencies and robust antibody staining

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Regulatory T cells (T_{regs}) are a subset of CD4+ T lymphocytes, characterised by high expression of FOXP3 transcription factor. T_{regs} are responsible for maintaining immune homeostasis and self-tolerance by suppressing the effector function of conventional CD4+ and CD8+ T cells, as well as inhibiting other immune cell subsets. Therefore, dysfunction or loss of T_{regs} can lead to a break in tolerance and drive autoimmunity. Gene engineering of patient T_{regs} for improved stability and function aims to provide a potentially curative treatment for severe autoimmunity in inflammatory bowel disease, rheumatoid arthritis, and more, while differentiating from current T_{reg} therapies.

To develop an engineered-T_{reg} cell therapy, we must first identify novel genes for T_{reg} stabilisation through a whole genome screening approach. To this aim, we have built a new capability for arrayed CRISPR screening in primary human T_{reg} cells that will validate hits arising from a whole genome pooled screen. We have developed an automation workflow that allows primary T_{regs} to be CRISPR-edited by Cas9-sgRNA ribonucleoprotein electroporation at scale in 384-well plates. To test the editing workflow, we targeted both neutral and control genes important to T_{reg} function, achieving ≥87% KO efficiency at all loci.

We then combined this editing workflow with a newly-developed 384-well flow-staining protocol that uses the iQue Flow Cytometer. We optimised a 10-colour flow panel that allows us to assess markers of T_{reg} stability and function, and achieved robust staining with no signal spill-over between channels.

Having achieved high editing efficiencies and generated reliable flow data, this capability has enabled arrayed CRISPR screening in primary T_{regs} for the first time in AstraZeneca (AZ). This new capability will be utilised as part of our cross-functional collaboration in AZ, with the aim to identify novel targets for an autologous *ex-vivo* CRISPR edited T_{reg} cell therapy in our future portfolio.