Development of analytical strategy to ensure production efficiency and consistency of a WT1-TCR immunotherapy

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Challenge:

Manufacturing of immunotherapies typically involves the use of viral vectors for the delivery of a CAR/TCR construct to patient T-cells. However, the use of patient specific starting material can lead to variability in transduction efficiency and product potency. Characterisation is therefore critical during and post-manufacture to ensure consistency and sufficient function.

Proposed solution:

We have investigated an advanced panel of in-process control and product release assays for retrovirally transduced WT1-T-cell-product during their activation from a manufacturing perspective (Fig. 1). T-cell activation was induced for efficient retroviral transduction. The developed assays include:

1. Immunometabolism profiling
2. Gene expression profiling
3. Evaluation of product potency using a label free cell index based assay in adherent cell lines

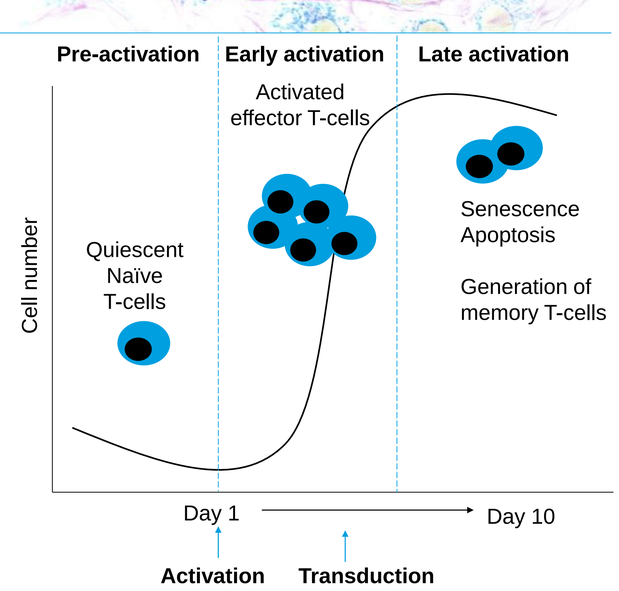

Fig.1 Schematic representation of T-cell transduction

1. Immunometabolic profiling

Aim:
We proposed to perform a quantitative assay to measure mitochondrial respiration, glycolysis and cell cycle analysis to identify the optimal time for WT1 γ-retroviral transduction during the manufacturing process.

Method:
Oxygen consumption rates (OCR, mitochondrial respiration), extracellular acidification (ECAR, glycolysis) and percentage of cells in G2 phase were measured in CD4/8+ human T-cells during activation.

Results:
1. Frozen material (Fig.2A):
- OCR significantly increased at 72h post-activation but dropped down at 96h.
- ECAR significantly peaked at 48h and levels of glycolysis remained high.
- Upregulation of metabolism correlated with the cells entering G2 phase (double DNA content, ready for mitosis).

2. Fresh material (Fig.2B):
- Results shown above were validated by analysing freshly isolated T-cells. Similarly, it was shown that metabolic upregulation was associated with T-cell proliferation.

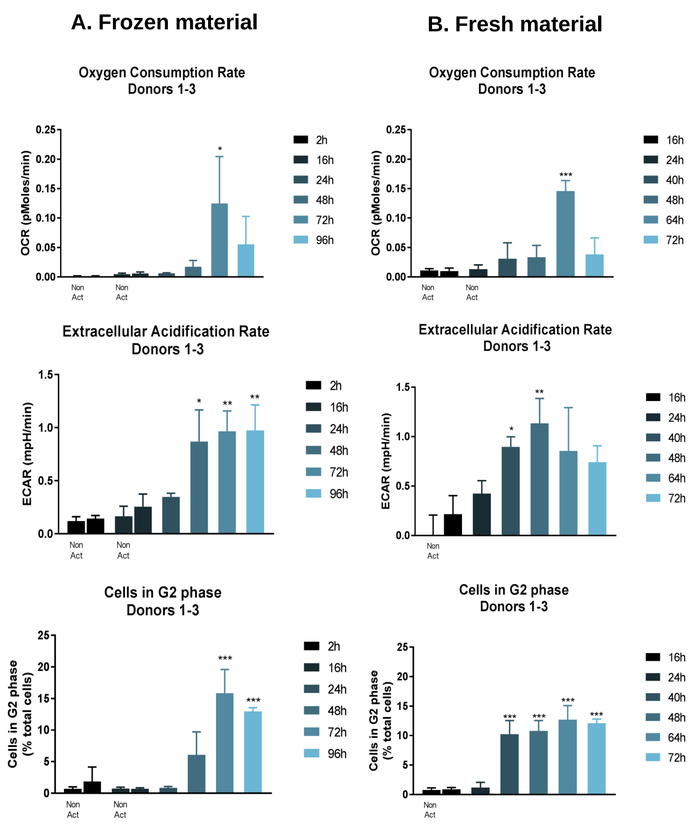

Fig.2 Metabolic activity and cell cycle analysis of frozen and fresh CD4/8+ T-cells after activation Data shown represents mean ± SD; one-way ANOVA followed by Tukey's post-test; * (p<0.05), **(p<0.01) and ***(p<0.001); n=3 donors per group.

Conclusion:
Results indicated that the assay could be used to predict the optimal time for WT1 γ-retroviral transduction in activated T-cells.

2. Gene expression profiling

Aims:
- To characterise gene expression profiles of T-cells during the manufacturing process.
- Using gene markers in an in-process control assay to inform on and aid the manufacturing process (e.g. time of activation, cell division and transduction).

Method:
Non-transduced T-cells (n=4) prior to activation (Day 1) and post-activation (Day 3, 6, 8 and 10) were run on a Fluidigm gene expression array for genes relevant to T-cell activation, proliferation, differentiation and polarisation.

Results:

1. PCA demonstrates clustering samples into stages of T-cell development (Fig. 1) – Day 1 samples at pre-activation stage, Day 3 at early activation, and Day 10 at late activation:

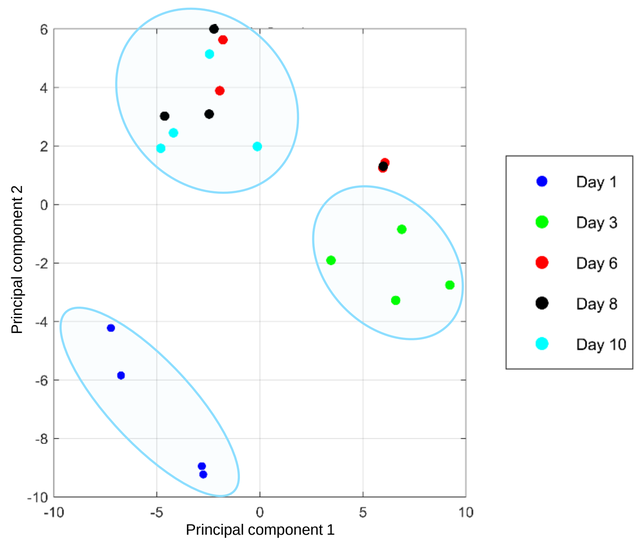

Fig.3 PCA shows clustering of samples by stages of T-cell development. Data shown represents T-cell samples collected on Day 1,3,6,8, and 10 of T-cell expansion for n=4 donors.

2. Examples of stage-specific gene hallmarks are shown in Fig.4:

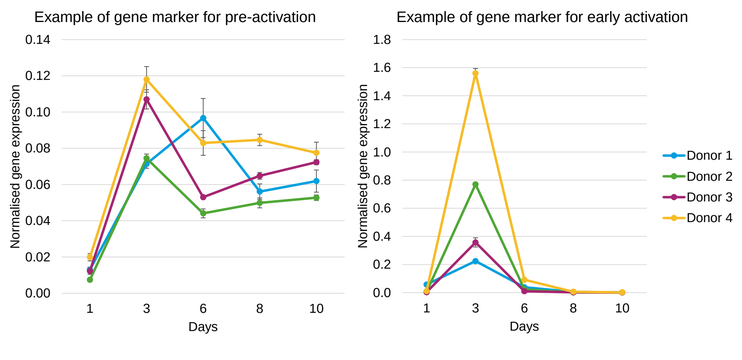

Fig.4 Examples of gene markers for pre-activation and early activation of T-cell development. Data shown represents mean expression ± SD on each day of expansion for n=4 donors. Student's t-test was used to select gene markers for each stage, $p<5.95\times10^{-4}$ corrected for 84 genes.

Conclusion:
19 gene markers were identified, defining specific stages of T-cell development and informing on the timing of critical steps during the manufacturing process (e.g. activation and cell division).

3. Evaluation of product potency using a label free cell index based assay in adherent cell lines

Aim:
We performed a label free potency assay based on the xCELLigence platform to replace the current method based on chromium release.

Method:
Target S-cell line was pulsed with the specific peptide of interest. TCR engineered effector cells (50% CD8+, 70% Dextramer+) were seeded at 8:1 and 2:1 effector:target ratios. Cell index was monitored over 4h (Figure 4.1). Control cell lines T2 and O (HLA-A2 negative) were used as positive and negative controls respectively (Figure 4.2).

Results:
1. Analysis of cell index on the xCELLigence platform in S Cell line. WT1 pulsed S-cell line showed faster killing (EC50=90min) at 8:1 compared to the 2:1 ratio (EC50=120min). Non transduced cells (NT) behave as target cells only (Fig.5).

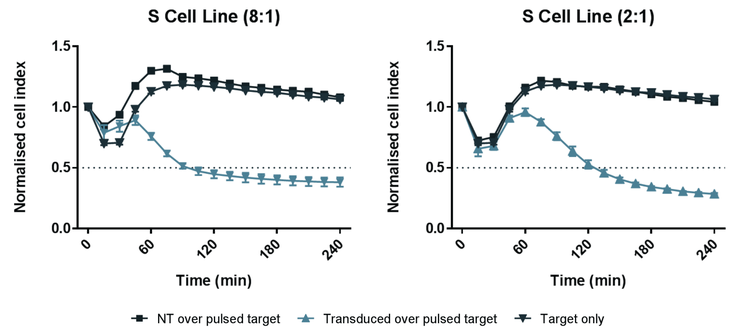

Fig.5 Evaluation of T-cell cytotoxicity in "S-cell line". Cell index measured every 15min. Three technical replicates per condition. P-value at EC50<0.0001.

2. WT1 pulsed T2 cell line showed specific killing. EC50 observed after 375min (6.25h) after target cell co-culture. However, no killing was observed in the O-cell line - very low expression levels of HLA-A2 on their surface. Therefore, TCR engineer cells are not able to recognize them (Fig.6).

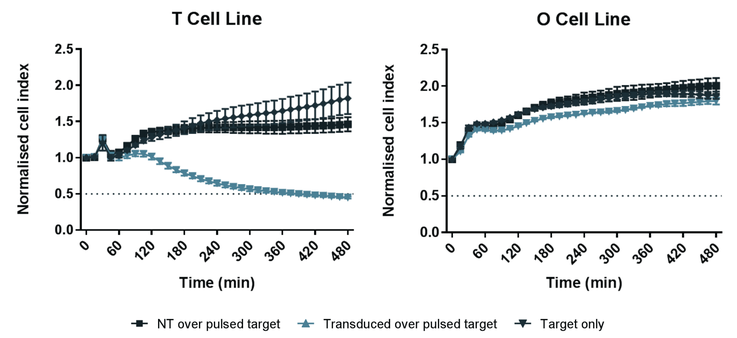

Fig.6 Evaluation of T-cell cytotoxicity in control T2 and O cell lines. Cell index measured every 15min. Three technical replicates per condition. P-value at EC50<0.0001.

Conclusion:
These results indicated that this platform can be used to measure the potency of the product and that it is a safer alternative to the chromium release assay.

Conclusions

- We have developed a suite of assays that could be used to monitor and control the manufacture of TCR-immunotherapies
- The set of analytical methods can be used to account for donor variability in starting material and minimises the risk of batch failure.

We work with Innovate UK

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