# The Achilles VELOS™ Process 2 boosts the dose of highly functional clonal neoantigen-reactive T cells for precision personalized cell therapies

Joseph Robinson[1], Amber Rogers[1], Daisy Melandri[1], Amy Baker[1], Anabel Ramirez Aragon[1], Sidra Nawaz[1], Michael Epstein[1], Shreenal Patel[1], Jennine Mootien[1], Andrew Craig[1], Satwinder Kaur-Lally[1], Hinal Patel[1], Andreas Schmitt[2], Farah Islam[3], Mariam Jamal-Hanjani[3], David Lawrence[4], Martin Forster[3], Samra Turajlic[2], Sergio A. Quezada[1], Katy Newton[1], Eleni Kotsiou[1]

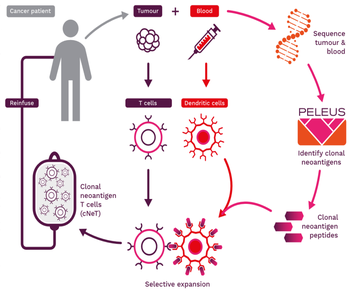
1) Achilles Therapeutics UK Limited, London, United Kingdom; 2) Royal Marsden NHS Foundation Trust, London, United Kingdom; 3) University College London Cancer Institute, London, United Kingdom; 4) UCLH and Barts NHS Trusts, London, United Kingdom; 5) Corresponding author – for further information please email: s.quezada@achillestx.com

## Introduction

Adoptive transfer of ex-vivo expanded Tumour-Infiltrating Lymphocytes (TIL) has shown promise in the clinic. However, the non-specific expansion of TILs and the lack of understanding of the active component of TIL has resulted in poor correlation between clinical response and dose as well as poor understanding of response and resistance mechanisms. The VELOS™ manufacturing process generates a precision and personalised treatment modality by targeting clonal neoantigens with the incorporation of an antigen-specific expansion step to enrich the product for these specificities. Achilles has developed a second VELOS™ process to boost the neoantigen-reactive cell dose while maintaining key qualitative features associated with function. Here we report the in-depth characterisation of clonal neoantigen-reactive T cells (cNeT) products expanded using the two VELOS™ processes.

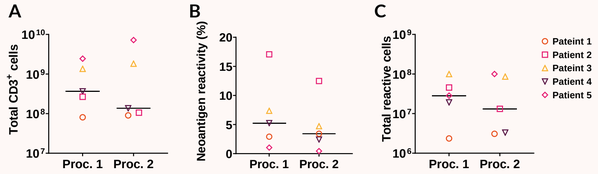
## Methods

- Matched tumours and peripheral blood from patients undergoing routine surgery were obtained from patients with primary NSCLC (n=3) or metastatic melanoma (n=2) (NCT03517917).
- TIL were expanded from tumour fragments in the presence of IL-2.
- Peptide pools, corresponding to the clonal mutations identified using the PELEUS™ bioinformatics platform, were generated.
- cNeT were expanded by co-culture of TIL with peptide-pulsed autologous dendritic cells.
- For VELOS™ Process 2 additional media supplementation was added throughout the process. Cell expansion was boosted at the end of the co-culture with an optimized stimulation cocktail.
- Neoantigen reactivity was assessed using our proprietary potency assay with peptide pool rechallenge followed by intracellular cytokine staining. Single peptide reactivities were identified using ELISpot and flow cytometric analysis for in-depth phenotyping of cNeT was performed.



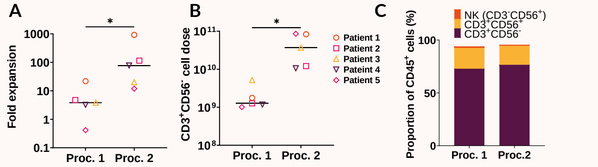

## Results

### Figure 1: Clonal neoantigen specific TIL can be identified following the culture of tumour fragments in VELOS™ Process 2



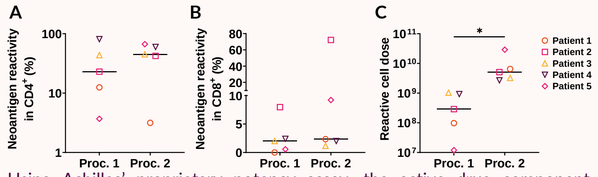

Following culture of tumour fragments with IL2, processes 1 and 2 yielded similar numbers of TIL (A; values scaled to tumour mass). Achilles' proprietary potency assay was used to identify the proportion of clonal neoantigen reactive cells within the TIL (B). The total number of clonal neoantigen reactive TIL was similar in processes 1 and 2 (C). Lines at median; n=5.

### Figure 2: VELOS™ Process 2 generates a 29 fold greater number of T cells




During the selective expansion phase of the VELOS™ process, Process 2 gave a greater fold expansion of T cells (A; lines at median) and an increase in total T cells generated (B; values scaled to tumour mass; lines at median). The majority of cells generated by both processes were $CD3^+CD56^-$ (C; bars show means). * p<0.05 one tailed Wilcoxon test, n=5.

### Figure 3: VELOS™ Process 2 generates a 18 fold greater number of cNeT



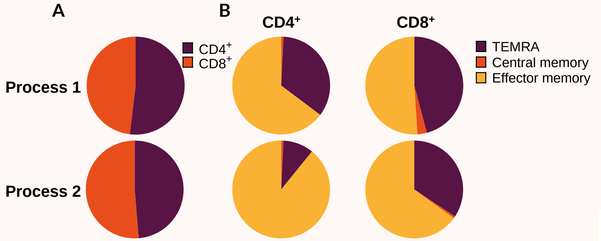

Using Achilles' proprietary potency assay, the active drug component (cNeT) was quantified for both $CD4^+$ (A) and $CD8^+$ (B) cells. No difference in the proportion of cNeT was observed between Process 1 and Process 2. The overall number of cNeT generated by Process 2 was significantly higher than was generated by Process 1. Lines at medians; * p<0.05 one tailed Wilcoxon test; n=5.

### Figure 4: VELOS™ Process 2 generates a product with multiple clonal neoantigen reactivities

| | Single peptide reactivities | | |
|---|---|---|---|
| Patient | Process 1 | Process 2 | Difference |
| 1 | 1 | 4 | +3 |
| 2 | 1 | 3 | +2 |
| 3 | 2 | 5 | +3 |
| 4 | 2 | 17 | +15 |
| 5 | No data | 18 | N/A |

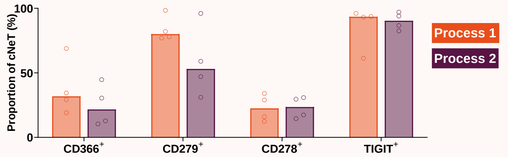
The number of individual clonal neoantigen reactivities was determined by ELISpot. VELOS™ Process 2 generated a product with reactivities to multiple clonal neoantigens without loss of reactivities compared to Process 1. For patient 5, insufficient cells were generated by Process 1 to carry out ELISpot.

### Figure 5: VELOS™ Process 2 generates a product made up of mainly $CD4^+$ and $CD8^+$ effector memory cells



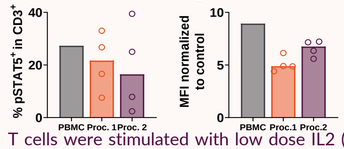

Phenotyping was carried out by flow cytometry at the end of the co-culture period. The VELOS™ processes generated products made up of both $CD4^+$ and $CD8^+$ cells (A; n=5). Products were primarily effector memory cells ($CD45RA^-CD197^-$) with some TEMRA cells ($CD45RA^+CD197^-$) and few central memory cells ($CD45RA^-CD197^+$) . The products of Process 2 had a lower proportion TEMRA cells compared to Process 1 (B; n=4). Pie charts show mean frequencies.

### Figure 6: cNeT from VELOS™ Process 2 express similar levels of immune checkpoint molecules



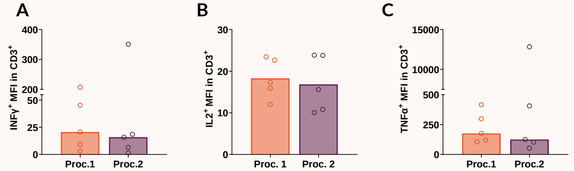

Restimulation with clonal neoantigen pareptide pools and staining for cytokine secreting cells enables phenotyping of the active drug component of the product of the VELOS™ process. cNeT from Process 2 showed no increase in immune checkpoint molecules compared to cNeT from Process 1. Bars at median; n=4.

### Figure 7: T cells from VELOS™ Process 2 retain sensitivity to IL2




T cells were stimulated with low dose IL2 (100IU/ml) and phosphorylation of STAT5 was measured by flow cytometry. Phosphorylation occurred in similar proportions of $CD3^+$ cells in both processes (A). Geometric mean fluorescence intensity (MFI) of pSTAT5 staining was also similar (B). Bars at median; n=4.

### Figure 8: T cells from VELOS™ Process 2 retain capacity to secrete cytokines




T cells were stimulated with a poly clonal stimulus (Staphylococcal Enterotoxin B) and cytokine production was measured using Achilles proprietary potency assay. $CD3^+$ cells from Process 1 and Process 2 generated similar amounts of INFγ (A), IL2 (B) and TNFα (C). Graphs show geometric mean fluorescence intensity (MFI) normalized to control; bars at median; n=5.

## Conclusions

- Achilles proprietary potency assay quantifies cNeT dose facilitating optimization of the VELOS™ process.
- VELOS™ Process 2 generates an increased cNeT dose compared to Process 1
- cNeT generated using VELOS™ Process 2 maintain key phenotypic features associated with function
- This proof of concept data supports the transfer of VELOS™ Process 2 to clinical manufacture for two first in human studies for treatment of solid cancer.

**References**

1: McGranahan N., et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 6280: 1463-1469 (2016)

2: Robertson J., et al. Adoptive cell therapy with tumour-infiltrating lymphocytes: the emerging importance of clonal neoantigen targets for next-generation products in non-small cell lung cancer. Immuno-oncology Technology. 3:1-7 (2019)

**Acknowledgements**

The authors would like to thank the participating patients and their families for donation of material used in this study.

**Disclosures**

This study was funded by Achilles Therapeutics UK Limited.