Achilles VELOS Process 2 generates a >10-fold improvement in cNeT dose over Process 1 with a highly potent polyclonal phenotype and has been successfully validated at GMP scale for clinical use in solid cancer

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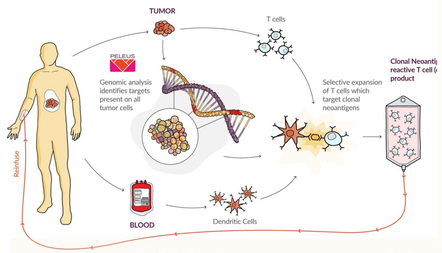
Introduction

Adoptive transfer of ex-vivo expanded Tumour-Infiltrating Lymphocytes (TIL) has shown promise in delivering durable responses in several solid tumour indications. However, characterisation of the active component of TIL therapy remains challenging due to the non-specific expansion of TILs in the manufacturing process. Achilles Therapeutics has developed ATL001, a patient-specific TIL-based product, manufactured using our VELOS™ manufacturing platform (Figure 1) that specifically targets clonal neoantigens, a subset of patient specific mutations present on all tumour cells. Process 1 of the VELOS™ manufacturing platform has successfully demonstrated the feasibility of generating clonal neoantigen reactive T cells (cNeT) products for the treatment of advanced NSCLC (CHIRON, NCT04032847) and recurrent or metastatic melanoma (THETIS, NCT03997474). Here we report on the successful GMP validation of VELOS™ Process 2 and demonstrate the generation of a significant dose boost of highly potent and reactive CD8+ and CD4+ cNeT for future clinical use with a comparable vein-to-vein time to Process 1.

Methods

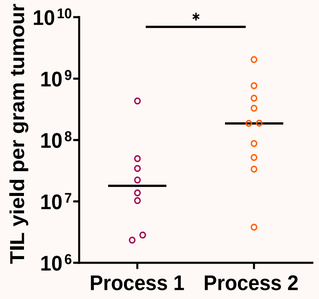
Figure 1: VELOS™ Process



- GMP Process 2 data was generated from tumour and blood samples procured at the time of surgery through our ethically approved non-clinical study (NCT03517917) in addition to excess material from clinical samples in NSCLC (n=8) or metastatic melanoma (n=2) patients.
- Process 1 GMP data was generated from manufactured products in eight patients dosed to date across THETIS (n=5) and CHIRON (n=3).
- TIL were isolated from tumour fragments in the presence of IL-2 and Dendritic Cells (DCs) generated from whole blood.
- Patient-specific peptide pools were synthesised, corresponding to the clonal neoantigen mutations identified using our proprietary PELEUS™ bioinformatics platform.
- cNeT were expanded by co-culture of TIL with peptide-loaded DCs.
- For VELOS™ Process 2 additional media supplements were added throughout the process. Cell expansion was boosted at the end of the co-culture with an optimized stimulation cocktail.
- cNeT reactivity was assessed using our proprietary potency assay with peptide pool rechallenge followed by intracellular cytokine staining. Product characterisation and phenotype was assessed by flow cytometric analysis.

Results

Figure 2: VELOS™ Process 2 generates higher TIL numbers following culture of tumour fragments

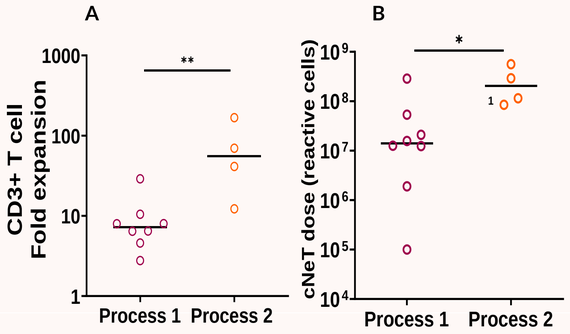


Generation of pre-REP TIL intermediates at GMP scale with VELOS™ Process 2 generated a 10-fold increase in TIL yield compared to VELOS™ Process 1 (Process 1: 18.2 x 10^6 TIL per gram vs. Process 2: 187.7 x 10^6 TIL per gram). Lines at median;

*p<0.05 two-tailed Mann Whitney test.

VELOS™ Process 1: n=8, VELOS™ Process 2: n=10,

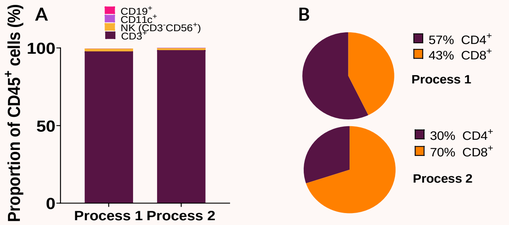
Figure 3: VELOS™ Process 2 delivers a >10-fold increase in cNeT doses compared to VELOS™ Process 1



TIL intermediates were used for the selective expansion step with peptide-loaded DCs. Process 2 resulted in a 55-fold increase in the total number of CD3+ T cells compared with a 7-fold increase in Process 1 (A; lines at median, ** p<0.005 two-tailed Mann Whitney test). The active drug component was quantified using Achilles' proprietary potency assay and showed a >10-fold increase with Process 2 yielding a median in cNeT dose of 203.5 x 10^6 compared with 14.2 x 10^6 in Process 1 (B; lines at median, * p<0.05 two-tailed Mann Whitney test).

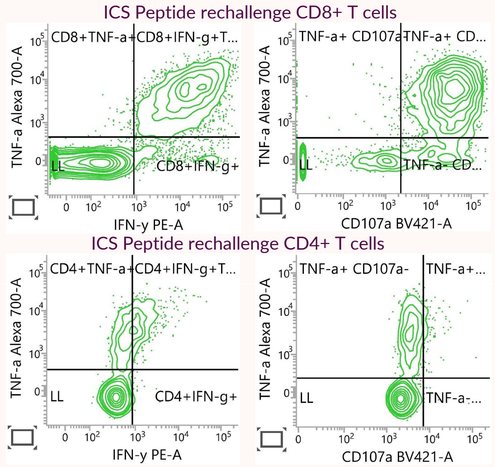
For both figures: VELOS™ Process 1: n=8, VELOS™ Process 2: n=4, [1]*Extrapolated to reflect minimum tumour volume for clinical procurement*

Figure 4: VELOS™ Process 2 generates a high purity CD3+ T cell product that retains both CD4+ and CD8+ T cell subsets



Identity and purity (A) were assessed in both infused products manufactured with Process 1 (n=8) and GMP validation runs with Process 2 (n=4). CD3+ T cell purity was comparable in both processes (97.8% vs. 98.6%) with low levels (<2%) of cell impurities. Final products contained a mix of both CD4+ and CD8+ T cells with a trend to more CD8+ T cells with VELOS™ Process 2 (B).

Figure 5: VELOS™ Process 2 generates a polyfunctional cNeT product at GMP scale



cNeT reactivity in CD4+ and CD8+ T cells was quantified by cytokine secretion in response to peptide rechallenge to determine the active component and further characterisation through measurement of CD107a (Figure 5). Comparison of cNeT reactivity (Figure 6 A) and T polyclonal stimulation with SEB (Figure 6 B) was comparable between VELOS™ Process 1 and Process 2. Figure 6 A and B, lines at median, no significance different, two-tailed Mann Whitney test).

Figure 6: VELOS™ Process 2 cNeT reactivity and polyclonal stimulation was comparable to VELOS™ Process 1

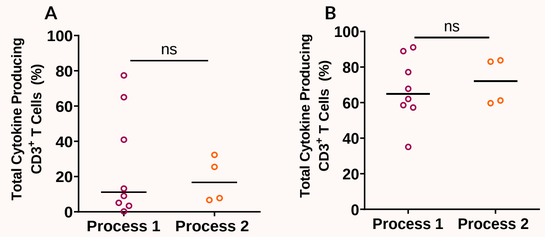
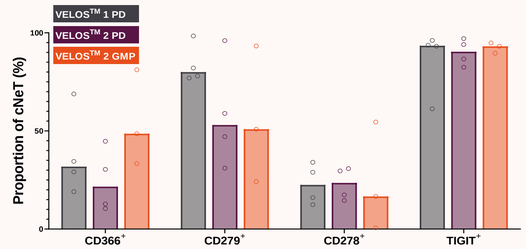


Figure 7: VELOS™ Process 2 express similar levels of immune checkpoint molecules to initial process development runs



Restimulation with clonal neoantigen pareptide pools and staining for cytokine secreting cells enables further characterisation of the active cNeT drug component (Figure 7). Immune checkpoint molecules were comparable between products manufactured with VELOS™ Process 2 at GMP scale (n=3) and small scale non-clinical process development batches generated with VELOS™ Process 1 and Process 2. Bars at median.

Conclusions

- We performed four VELOS™ Process 2 runs at scale to GMP standards and all batches successfully met all QC release criteria.
- This data demonstrates the ability to generate significantly higher cNeT doses at GMP scale using VELOS™ Process 2 and accurately identify the active drug component.
- Successful GMP Validation of Process 2 supports transfer into a clinical setting for the treatment of advanced NSCLC and melanoma in two first-in-human studies with potential utility across a variety of solid tumours.