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Next generation clonal neoantigen targeting T cells, generated using the PELEUS™ bioinformatics platform and the VELOS™ manufacturing method show superior reactivity and phenotypic characteristics than classical TIL products

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Adoptive transfer of tumor infiltrating lymphocytes (TIL) has generated objective clinical responses in patients with advanced metastatic cancers. Therapeutic exploitation of neoantigens as targets can potentially lead to safer and more effective treatment modalities with reduced toxicities. The Achilles Therapeutics trial [NCT03517917](#) enabled the acquisition of matched tumor specimens and peripheral blood samples from patients undergoing routine surgery and facilitated the development of the proprietary VELOS™ manufacturing process, generating a personalized clonal neoantigen specific T cell product. An in-depth characterization of T cells expanded with the VELOS™ process was performed and compared to a standard TIL product. Samples were obtained from patients with primary NSCLC or metastatic melanoma. TIL were expanded from tumor fragments after dissection in the presence of IL-2. Peptide pools corresponding to the clonal mutations that were identified using the PELEUS™ bioinformatics platform were used to pulse dendritic cells (DC) generated from peripheral blood monocytes from each patient. Clonal neoantigen specific T cells (cNeT) were expanded using the VELOS™ process by co-culture of TIL with the peptide-pulsed autologous DC. As a comparison, TIL were expanded with a rapid expansion protocol (REP-TIL) in the presence of allogeneic feeders, anti-CD3 antibody and high-dose IL-2. Intracellular cytokine staining was performed following rechallenge with individual peptide pools encoding the clonal mutations. Single peptide reactivities were identified using ELISPOT and extended flow cytometric analysis of markers associated with T cell fitness or dysfunction was performed to phenotypically characterize the cNeT, TIL and REP-TIL. Analysis of the immune cell composition showed that cNeT, TIL and REP-TIL have similar CD3⁺ T cell content (median cNeT 90.2%, TIL 87.3%, REP-TIL 95%, n=6) and are composed of CD4⁺ and CD8⁺ T cells (median CD4:CD8 ratio- cNeT 11.1, TIL 2.03 and REP-TIL 4.7, n=6). cNeT showed superior clonal neoantigen specificity compared to TIL or REP-TIL. The proportion of CD3⁺ T cells responding to clonal neoantigen rechallenge was increased in cNeT (median 24.3%) compared to TIL (median 0.6%) and REP-TIL (median 1.8%) (n=5). The VELOS™ process incorporating the PELEUS™ platform for prediction of clonal neoantigens generates T cell products enriched for clonal neoantigen reactivities and superior phenotypic characteristics compared to conventional TIL. The VELOS™ process is currently being used to manufacture cNeT for two first-in-human studies including NSCLC and melanoma patients ([NCT04032847](#), [NCT03997474](#)). **Ethical approval:** The samples for the study were collected under an ethically approved protocol ([NCT03517917](#)).