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Establishing a scalable manufacturing process for genetically modified γδ T cells for preclinical research

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Immunotherapy has transformed modern oncology by enabling selective activation of the immune system against tumor cells. γδ T cells, characterized by MHC-independent antigen recognition and rapid cytotoxicity, represent a promising platform for allogeneic CAR-T cell therapy and off-the-shelf applications. The aim of this study was to establish a scalable manufacturing process for the cultivation and genetic modification of Vγ9Vδ2 T cells for cancer therapy.

Vγ9Vδ2 T cells were expanded from peripheral blood mononuclear cells of healthy donors using zoledronate in combination with varying concentrations of IL-2 and IL-15. Lentiviral transduction was performed to engineer expression of a second-generation CD19 CAR construct containing a CD34-derived C6 epitope in its hinge region, enabling magnetic enrichment using clinical-grade CD34 microbeads and the LP34 process on the GMP-compliant CliniMACS Prodigy platform. Phenotypic analysis of the cell subpopulations was conducted by flow cytometry.

The expansion of γδ T cells proved to be highly donor-dependent, making it challenging to establish a reproducible expansion protocol yielding consistent purity. While the addition of IL-15 consistently induced the strongest expansion in γδ T cells, starting frequencies ranged from 0.9 % to 5.03 % at day 0 and resulted in final γδ T cell proportions between 10.39 % and 58.64 % at day 14. Despite low starting frequencies of γδ T cells, αβ T cells and NK cells frequently predominated due to their high initial abundances at day 0 (αβ T cells ~55–64 % and NK cells ~5–16 %) and substantial residual expansion, resulting in final proportions at day 14 ranging from 4.77 % to 90.53 % for αβ T cells and from 16.28 % to 66.67 % for NK cells, depending on donor and culture conditions. Efficient transduction was achieved using Vectofusin-1, spinoculation, and lentiviral pseudotyping with the baboon endogenous retrovirus envelope protein. The LP34 process enabled a high purity of CAR-positive γδ T cells (90.44 %, based on BFP reporter expression), but resulted in a limited yield of 28.74 %. Functionally, the CAR-modified cells demonstrated increased cytotoxicity against CD19-positive target cells in vitro, achieving killing rates of approximately 80–90 % at low effector-to-target ratios, whereas untransduced T cells remained limited in their cytotoxic capacity, with around 28 % target cell killing at a 1:1 ratio.