Overcoming challenges in γδ T cell-based immunotherapy: A new transduction method for engineering CAR γδ T cells

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Introduction

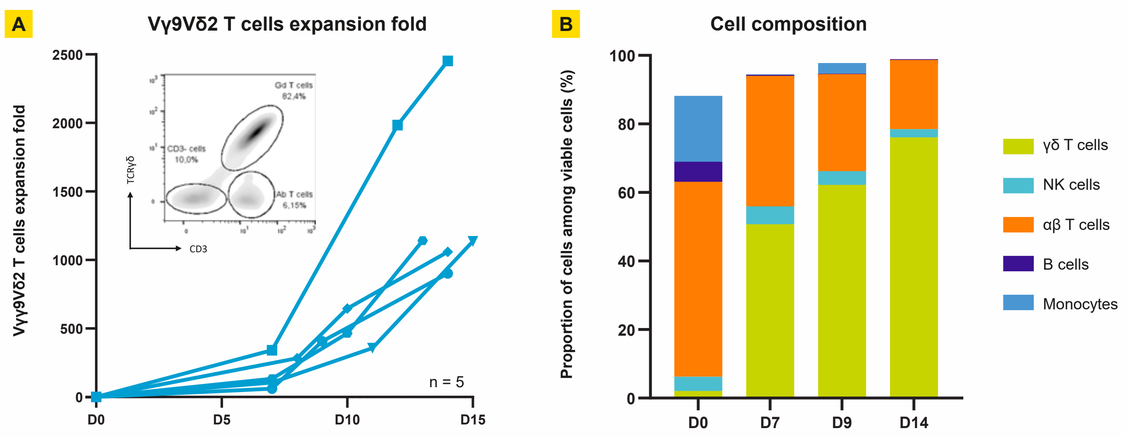
γδ T cells are promising effector cells for adoptive immunotherapy. Thanks to their MHC-independent antigen recognition and the detection of stress ligands[1,2], they are suitable for allogeneic clinical interventions. Vγ9Vδ2 T cells, the main population in human peripheral blood, have the advantage of naturally recognizing malignant cells, especially those of hematopoietic malignancies[3] by different mechanisms, such as sensing the upregulation of the mevalonate pathway happening in cancer cells[4]. This mechanism can be used to successfully expand these cells with Zoledronate and cytokines[5]. Moreover, their safety has already been demonstrated in both autologous[6,7] and allogeneic[8] clinical trials for hematological and solid tumors. However, genetic modification of γδ T cells has been shown to be challenging due to inefficient transduction. To enhance their natural cytotoxicity, a suitable engineering method is thus needed. Here, we have established an easily scalable protocol to produce CAR Vγ9Vδ2 T cells with a high transduction efficiency combined with a stable final phenotype.

Results

1 γδ T cells are efficiently expanded with a feeder-cell free method

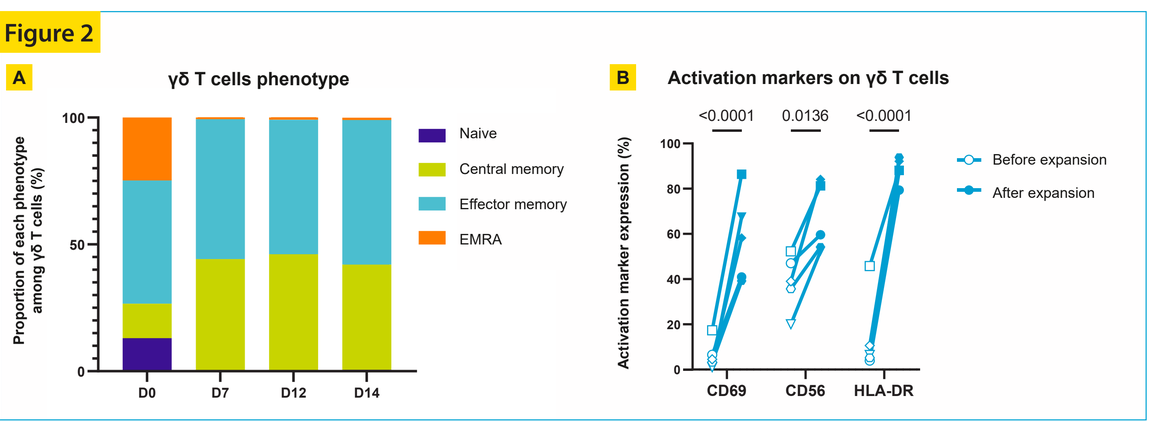
We activated and expanded γδ T cells from peripheral blood mononuclear cells (PBMC) using Zoledronic acid 5µM, IL-2 100 IU/ml and IL-15 100 IU/ml in TexMACS™ medium in the presence of human AB serum. We monitored the cellular composition, the phenotype and the activation profile by flow cytometry during two weeks of culture. We expanded Vγ9Vδ2 T cells from 5 donors at least 1000 times, reaching a purity above 75%. The final product consisted largely of γδ T cells and NK cells (Figure 1).

Figure 1



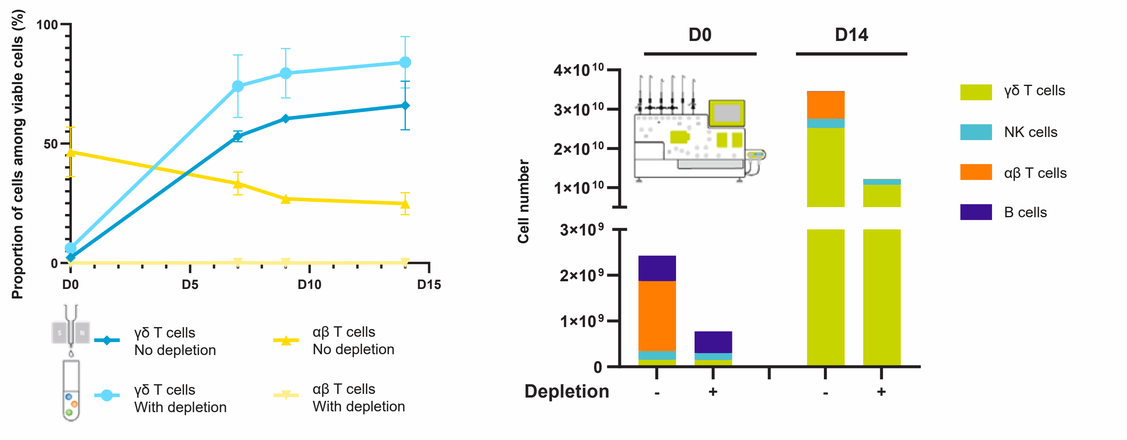
In all donors, the γδ T cells showed a memory phenotype (Figure 2A), and a high expression of the activation markers CD69, CD56 and HLA-DR at the end of the cultivation process (Figure 2B).

Figure 2



We performed αβ T-cell depletion using magnetic beads. The depletion was then performed with LS columns (Figure 3A) or on the CliniMACS Prodigy (Figure 3B). A depletion of at least 4 logs was reached with both methods. γδ T cells could then be expanded from the PBMCs.

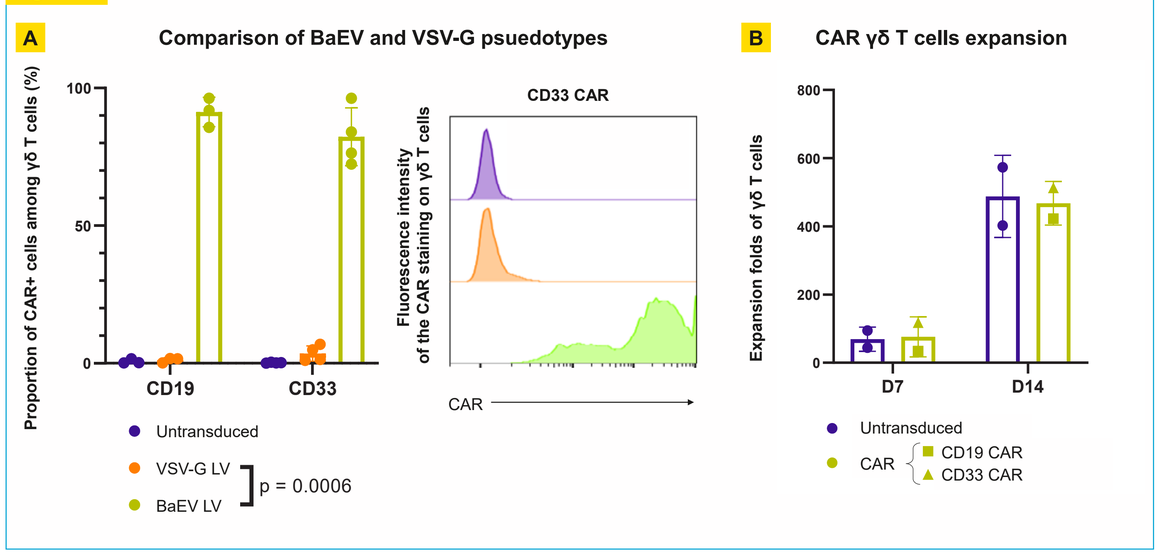
Figure 3



2 BaEV-pseudotyped lentiviral vector yields a high transduction efficiency of γδ T cells at a low MOI

We compared the VSV-G pseudotype with BaEV at a MOI of 0.5 with CD19 CAR and CD33 CAR constructs. Vectofusin®-1 was used at a concentration of 10 µg/ml to enhance the transduction efficiency of the BaEV pseudotype. Vectofusin-1™ did not increase transduction efficiency with VSV-G (data not shown). CAR expression and the number of γδ T cells were determined 7 days after transduction. The combination of the BaEV pseudotype and Vectofusin®-1 resulted in a 86.1% ± 9.4% transduction efficiency, which was significantly superior to VSV-G transduction efficiency for both CAR constructs tested (Figure 4A, p = 0.0006). The median fluorescence intensity was similarly higher with the BaEV pseudotype. The transduction did not impact the γδ T cell expansion (Figure 4B).

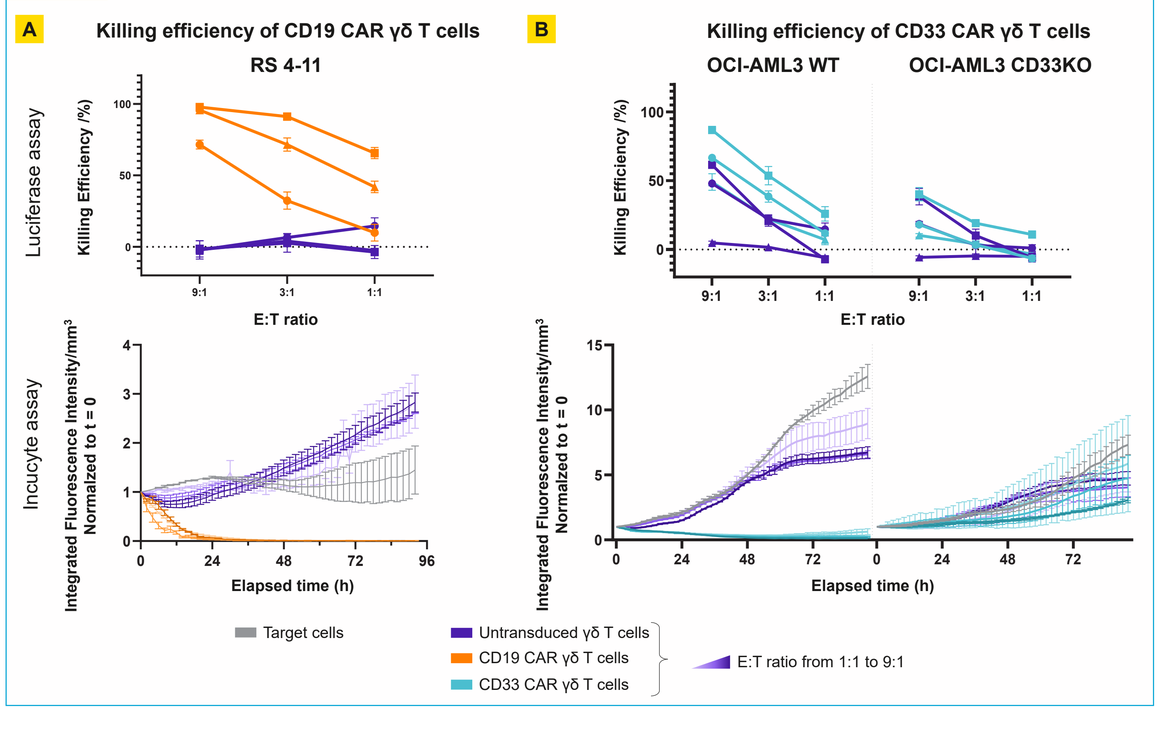
Figure 4



3 CAR γδ T cells efficiently kill hematological tumor cell lines *in vitro*

We tested the killing efficiency of γδ T cells after αβ T-cell depletion, transduction and further expansion with our protocol. The untransduced and CD19 or CD33 CAR γδ T cells were co-cultured with RS4-11 or OCI-AML3 WT and OCI-AML3 CD33 KO tumoral cell lines expressing GFP and Luciferase reporters. After 4h of co-culture, the Luciferase expression was measured with the ONE-Glo™ Luciferase Assay System (Promega) as a marker of the number of target cells. The growth of the target cells was monitored in parallel in the Incucyte® S3 System (Sartorius). CD19 CAR γδ T cells, but not untransduced γδ T cells, efficiently killed RS4-11 cells in both systems (Figure 5A). Similarly, CD33 CAR γδ T cells killed OCI-AML3 WT cells better than untransduced γδ T cells. The superior killing was only observed with the CD33 CAR and not with CD33 KO cells (Figure 5B).

Figure 5



Conclusion

- Vγ9Vδ2 T cells can successfully be expanded by a feeder cell-free protocol after αβ T cells depletion.
- The BaEV pseudotype combined with Vectofusin®-1 results in a high CAR transduction efficiency, without loss of expansion potential.
- Transducing Vγ9Vδ2 with CAR constructs enhances their natural cytotoxicity.
- This method is suitable for clinical production in a closed system such as the CliniMACS Prodigy®.

References

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