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Simplifying Progress

Overview of CAR-T Cell Generation Using Optimized Plasmid Design and Lentiviral Vector Production

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1. Introduction

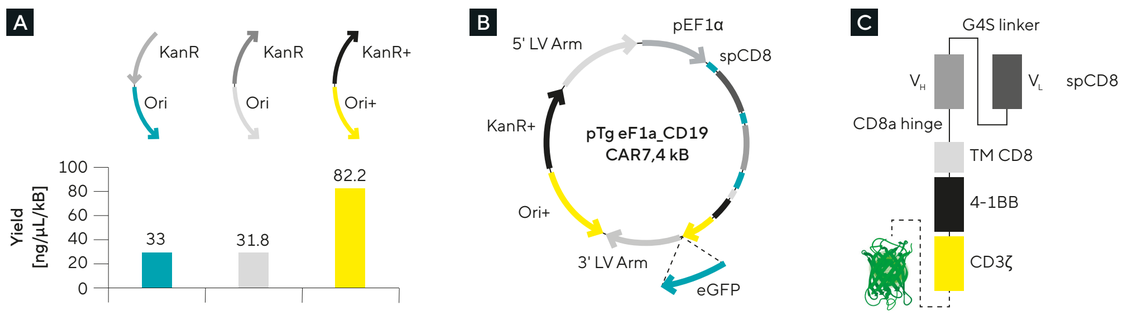
Gene modified cell therapy such as CAR-T cells is one of the most promising advanced therapy medicinal products (ATMP) to fight against cancer. These therapies typically use lentiviral vectors to transfer a gene of interest and modify patient's cells. All the raw material used during the lentiviral vector manufacturing must be carefully selected based on different parameters such as quality, performance, supplier, etc.

In this project, we generated CAR-T cells based on different plasmid constructs expressing a fusion protein CAR to track transduction efficiency. We first built different plasmid constructs using the plasmid engineering service from Polyplus, then encapsulate this gene of interest using a lentiviral vector produced with FectoVIR®-LV, transduce CD4+ CD8+ T cells from peripheral blood with this lentiviral vector, expand them for 12 days and finally test the efficiency of the transduced CAR-T cells using a killing assay.

2. Plasmid Design

We used our e-Zyvec® DNA assembly technology to modulate and optimize different part of the plasmids. First, several combination of bacterial genetic features (Origin of replication—Ori and antibiotic resistance—KanR) were tested to improve plasmid amplification rate. Here is shown that our optimized Ori+ (yellow) and KanR+ (black) casettes increase plasmid yield by almost 3 folds (A). Then we modularly built plasmids encoding various versions of a CD19-CAR differing by the presence or not of a eGFP reporter (C) or the use of different linkers (IRES, T2A or G4S peptide) between CAR and eGFP (not shown). For the purpose of the study, 5 plasmids were generated simultaneously in just under 4 weeks from design to plasmid sequencing.

Figure 1: *Plasmid Optimisation With Different Bacterial Backbones Using e-Zyvec® DNA Assembly Technology and Schematic Representation of the CD19 CAR LV pTransgene and of the Obtained CD19 CAR*

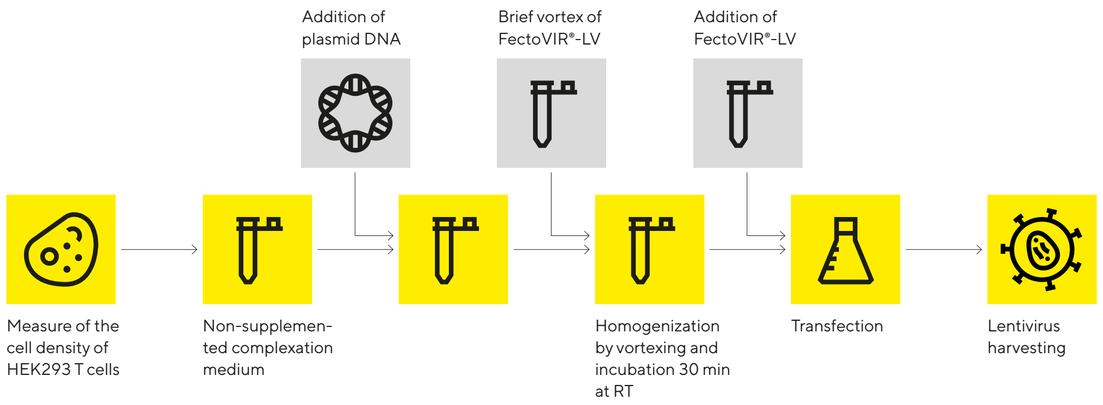


Note. (A) Plasmid yield obtained with different bacterial backbones. (B) Schematic representation of the CD19 CAR LV pTransgene. Features: 5' LV Arm contains the Rous Sarcoma Virus promoter, HIV-1 LTR and Psi sequence, RRE and cPPT/CTS. The Elongation-Factor 1α promoter allows transcription of the CD19 CAR. The latter is composed of the CD8 signal-peptide, the light and heavy chains of the CD19 single-chain variable fragment linked with G4S peptide, the hinge and trans-membrane domain of CD8, the intracellular signaling domains 4-1BB and CD3ζ. And for 3 constructs, there is the enhanced Green Fluorescent Protein linked with IRES, T2A or G4S peptide. The 3' LV arm contains HIV-1 ΔU3LTR and SV40-PA signal. Finally, there are our optimized Origin or replication and optimized Kanamycin resistance cassette. (C) Schematic representation of the obtained CD19 CAR.

3. Lentiviral Vector Production and Titration

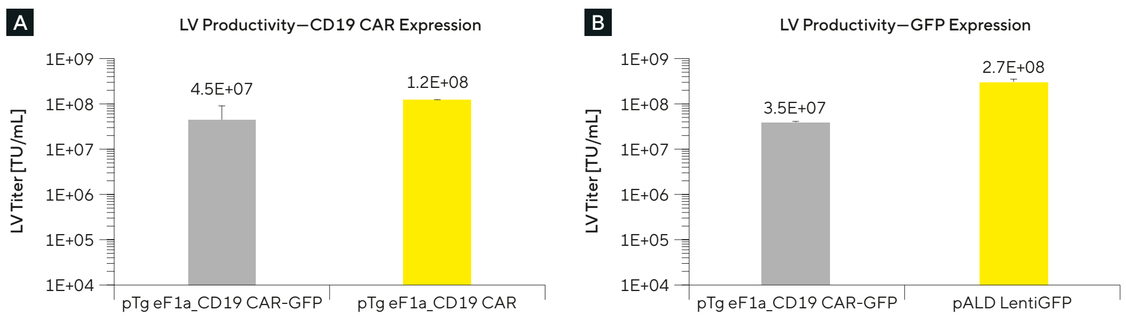
Lentiviral vectors (LV) were produced using FectoVIR®-LV and a 4-plasmid system in 125 mL shake flask with HEK293 T cells cultivated in suspension. The transfection step has been performed using the recommended conditions and LV were harvested 72 hours post-transfection.

Figure 2: *FectoVIR®-LV Protocol for Lentivirus Production*



Isolated human CD4+ and CD8+ T cells from one donor were activated with TransAct™ T cell reagent on day 0 and were transduced on day 1 with different lentiviral particles with Synperonic F108 and cultivated for 5 days. Expression of CD19 CAR and GFP was analyzed by flow cytometry on day 6.

Figure 3: *FectoVIR®-LV Demonstrates a High Productivity for CD19 CAR-GFP, GFP and CD19 CAR Lentiviral Vector Manufacturing*

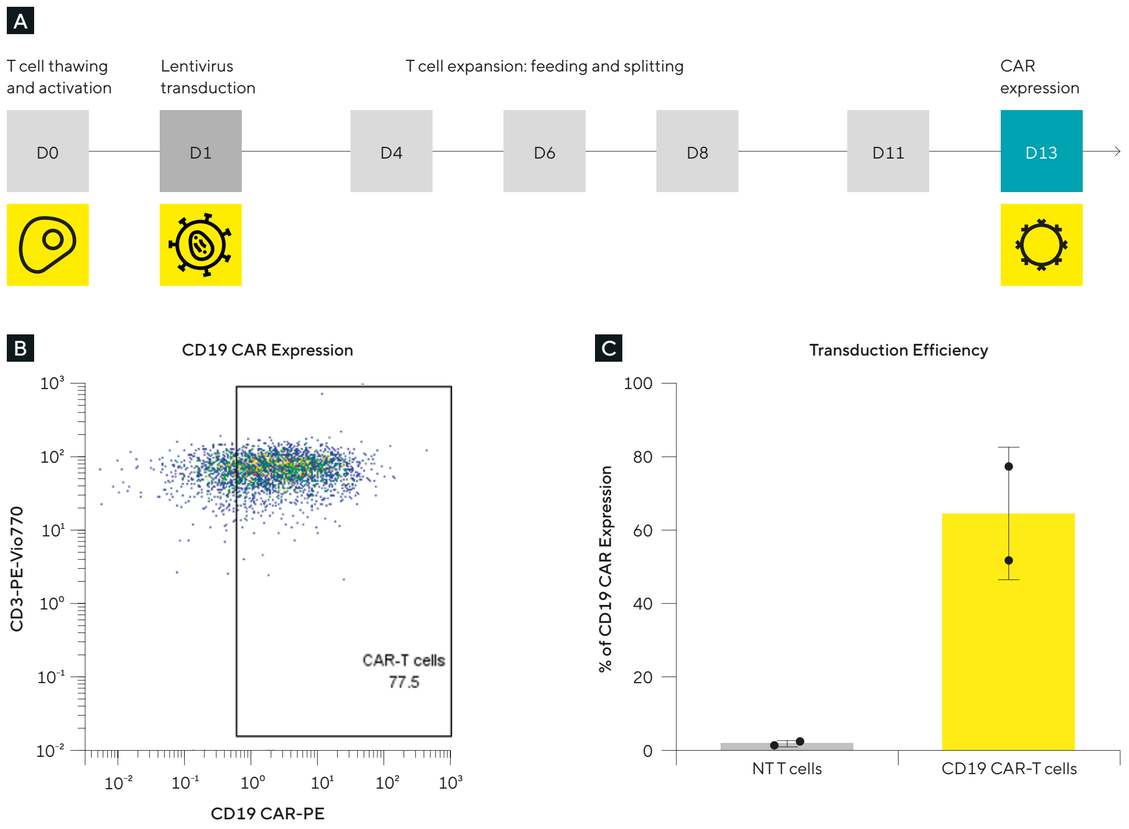


Note. Functional titers were measured using (A) GFP expression or (B) CD19 CAR expression on transduced CD4+ CD8+ T cells.

4. CD19 CAR-T Cell Generation

Isolated human CD4+ and CD8+ T cells from peripheral blood from two donors were activated with TransAct™ T cell reagent on day 0 and were transduced on day 1 with lenti-CD19 CAR viral particles (MOI 10) with Synperonic F108 and cultivated for 12 days (A). Expression of CD19 CAR was analyzed by flow cytometry on day 13 (B and C).

Figure 4: *CD19 CAR-T Cell Expansion and Evaluation of the Transduction Efficiency*

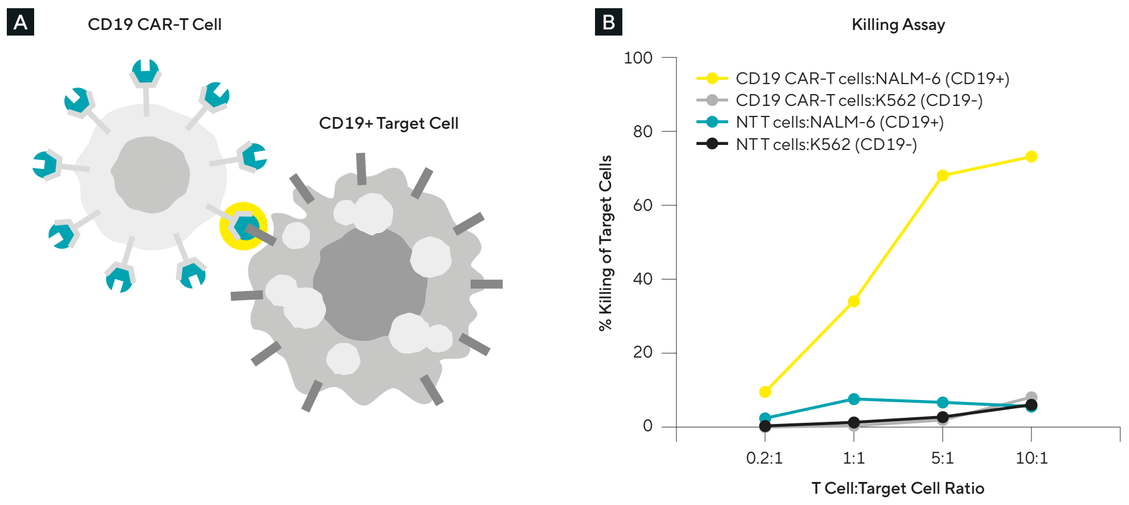


Note. (A) Workflow of T cell expansion, (B) Dot plot of transduction efficiency of one representative donor and (C) CD19 CAR expression on day 13 on non-transduced (NT) T cells and transduced T cells (n = 2 donors).

5. Efficient Killing of Target Cells

Following cognate antigen recognition, CAR-T cells should be able to kill the antigen-bearing tumor cell. To mimic this and to verify the functionality of the generated CD19 CAR-T cells, we used the NALM-6 cell line, which expresses the CD19 antigen and the K562 cell line as negative control, which does not express the CD19 antigen.

Figure 5: *CD19 CAR-T Cell Killing of Antigen Positive Target Cells*



Note. (A) CAR-T cells and target cells interaction in a co-culture assay and (B) thawed CAR-T cells or non-transduced T cells were co-cultivated with CellTrace Violet-labeled NALM-6 or K562 cells in 0.2:1, 1:1, 5:1 and 10:1 effector to target ratios. After 24 hours, killing of target cells by CD19 CAR-T cells or non-transduced T cells was assessed by PI+ CellTrace Violet+ target cells.

6. Conclusion

The data presented demonstrate the capacity of FectoVIR®-LV in combination with plasmids generated from e-Zyvec® DNA assembly technology to produce high yield of potent lentiviral vectors. Here with these lentiviral vectors, we've generated functional CD19 CAR-T cells which are capable to kill antigen-bearing tumor cells.