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Simplifying Progress

Innovative Lipid-Based Delivery Solution: Unlocking New Opportunities in Manufacturing Genetically Modified Immune Cell Therapies

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Introduction

Gene-modified cell therapies, such as engineered T cells or Natural Killer (NK) cells expressing chimeric antigen receptors (CARs), are among the most promising treatments for cancer and autoimmune diseases. Traditionally, these therapies rely on lentiviral vectors to transfer the gene of interest; however, this approach is associated with lengthy manufacturing timelines, high costs, and potential safety risks. To overcome these limitations, our research focuses on lipid-based solutions as a promising alternative for producing CAR-T and CAR-NK cells.

In this study, innovative lipid-based solution LipidBrick® Cell Ready was employed first for transient protein expression with the production of functional *ex vivo* mRNA-based CD19 CAR-T cells and then for stable cell modification through the delivery of genetic engineering tools such as CRISPR using Cas9 mRNA and sgRNA targeting the TRAC gene. Optimization of T cell transfection was initially performed in 96-well plates before scaling up to a T25 flask. To demonstrate the versatility of this solution, diverse nucleic acid payloads (mRNA, plasmid DNA and nanoplasmid) and various cell types (T cells, NK cells, monocyte-derived dendritic cells and hematopoietic stem cells) were successfully transfected with our LipidBrick® Cell Ready reagent. Finally, we've shown that our ready-to-use solution LipidBrick® Cell Ready could be efficient to generate functional CD19 CAR-NK cells.

1. LipidBrick® Cell Ready: An Innovative Lipid-Based Delivery Solution

LipidBrick® Cell Ready is revolutionizing the field of gene-modified cell therapy by offering a ready-to-use lipid-based reagent that eliminates the need for specific equipment or additional consumables. Additionally, its simple 2-step protocol is designed for ease of use, making it suitable for a wide range of applications, from HTS in well-plates to therapeutic gene editing of cells at bioreactor scale. This simplicity and versatility make LipidBrick® Cell Ready a cost-efficient solution, empowering researchers and developers to advance their projects without the burden of complex procedures or high investment costs.

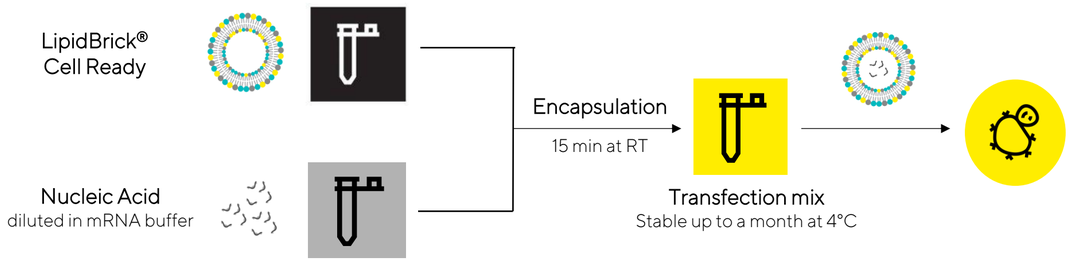

Figure 1: Simple 2-step mixing protocol to encapsulate mRNA into preformed lipid-based nanoparticles LipidBrick® Cell Ready.

2. CD19-CAR Cell Generation

Isolated human CD3+ T cells from peripheral blood were activated with TransAct™ T cell reagent on day 0 and expanded in CellGenix® GMP TCM media with a cocktail of CellGenix® cytokines (IL-7 and IL-15). On day 2, T cells were transfected with CD19 CAR mRNA with LipidBrick® Cell Ready in a 6-well plate. Our workflow resulted in high transfection efficiency while maintaining high cell viability when delivering CD19 CAR mRNA into both CD4+ and CD8+ T cells.

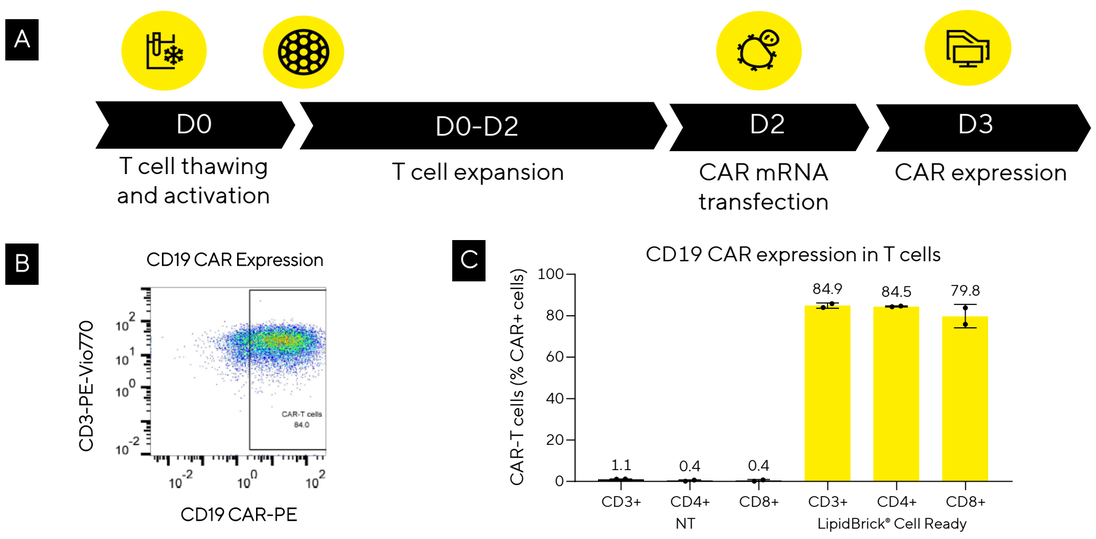

Figure 2: *(A) Workflow of T cell expansion, transfection and evaluation of the transfection efficiency by flow cytometry, (B) dot plot of CD19 CAR expression on transfected T cells (one representative donor) and (C) CD19 CAR expression on day 3 on non-transfected (NT) and transfected T cells with LipidBrick® Cell Ready with 0.8 µg CD19 CAR mRNA/million cells* (n=2 donors).

3. Efficient Killing of Target Cells

Following cognate antigen recognition, CD19 CAR-T cells should be able to kill the antigen-bearing tumor cell. To mimic this and to verify the functionality of the generated *ex vivo* mRNA-based 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. Effective killing of CD19+ tumor cells was achieved with the CD19 CAR-T cells.

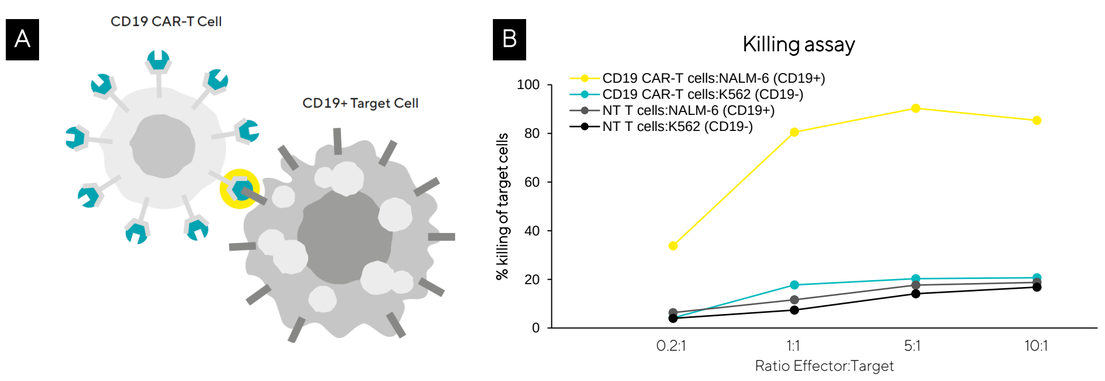

Figure 3: *(A) CAR-T cells and target cells interaction in a co-culture assay and (B) freshly transfected CD19 CAR-T cells or non-transfected T cells were co-cultivated with CellTrace Violet-labeled NALM-6 or K562 cells in 0.2:1, 1:1, 5:1 and 10:1 effector to target ratios. After 24 hours, killing of target cells by ex vivo mRNA-based CD19 CAR-T cells or non-transfected T cells was assessed by PI+ CellTrace Violet+ target cells.*

4. High Performance in Gene Editing

For the knock-out (KO) of the T-cell receptor (TCR), activated CD3+ T cells were transfected on day 3 with Cas9 mRNA and TRAC sgRNA with LipidBrick® Cell Ready. Expression of TCR was analyzed by flow cytometry 2 and 3 days after the transfection. High KO rates of the TCR was achieved while maintaining excellent cell viability.

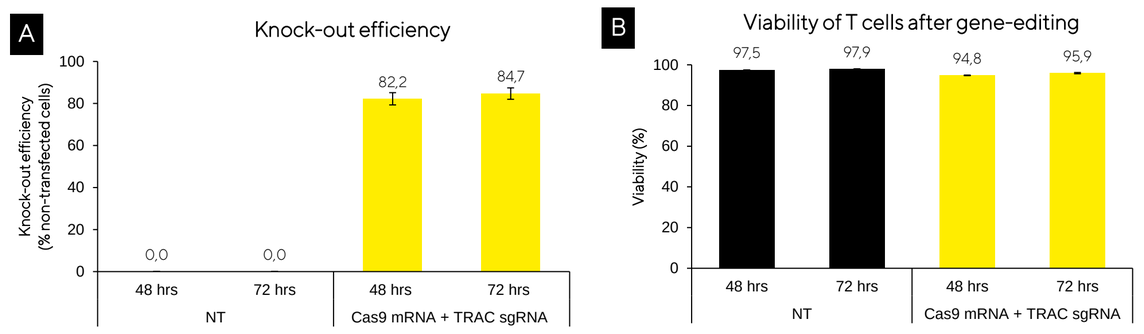

Figure 4: *(A) K/O efficiency and (B) viability of non-transfected (NT) or T cells transfected with LipidBrick® Cell ready with 0.27 µg Cas9 mRNA/millions cells and 1.44 µg TRAC sgRNA /millions cells after 48 and 72 hours of transfection.*

5. Scalability

Isolated human CD3+ T cells from peripheral blood were activated with TransAct™ T cell reagent on day 0 and expanded in 4Cell® Nutri-T GMP media with IL-2. On day 3, T cells were successfully transfected with GFP mRNA with LipidBrick® Cell Ready in a T25 flask.

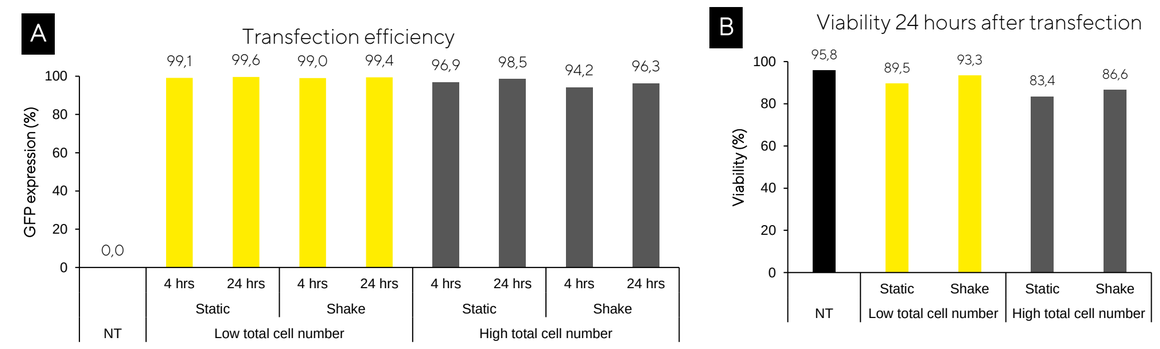

Figure 5: *(A) GFP expression and (B) viability 4 and 24 hours after transfection for NT cells and transfected T cells in a T25 flask with or without agitation at low or high total number of cells transfected/flask (5.4 or 27.8 millions cells/flask) with LipidBrick® Cell Ready with 0.27 µg GFP RNA/million cells.*

6. Versatility

To demonstrate the versatility of this solution, human activated T cells were successfully transfected with LipidBrick® Cell Ready and with GFP plasmid DNA or GFP nanoplasmid. Then various cell types including T cells, NK cells, monocyte-derived dendritic cells (MoDCs) and HSCs were transfected with GFP coding mRNA with LipidBrick® Cell Ready. High GFP expression was achieved in all cell types tested.

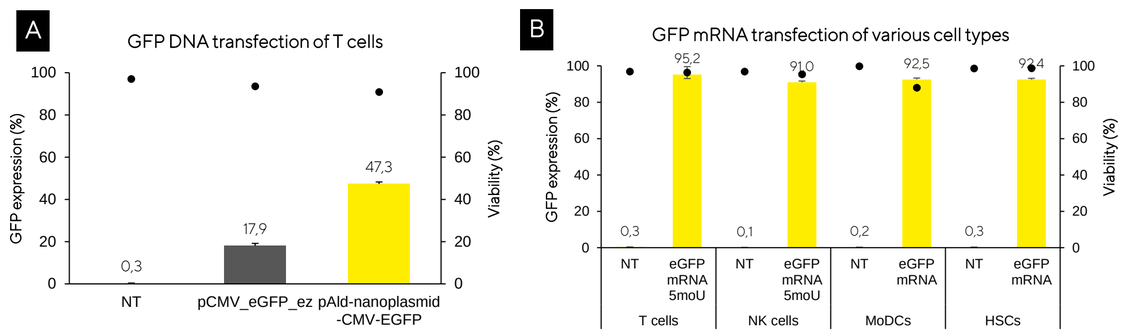

Figure 6: (A) *GFP expression in T cells one day after transfection for NT control cells and transfected T cells with either the pCMV_eGFP_ez plasmid DNA or the pALd-nanoplasmid-CMV-EGFP (0.53 µg GFP DNA per million cells). (B) GFP expression on various cell types one day after transfection with LipidBrick® Cell Ready with GFP coding mRNA modified or not with 5-methoxyuridines (5moU; 0.4 µg mRNA/million cells for T and HSC, 0.53 µg mRNA/million cells for NK cells and 1.06 µg mRNA/million cells for MoDCs).*

Isolated human CD56+ NK cells from healthy donors were activated and expanded for 10 days in CellGenix® SCGM media and a cocktail of CellGenix® cytokines (IL-2, IL-18 and IL-21) prior to transfection. NK cells were transfected with CD19 CAR mRNA with LipidBrick® Cell Ready in a 6-well plate. Effective killing of CD19+ tumor cells was achieved with the *ex vivo* generated CD19 CAR-NK cells.

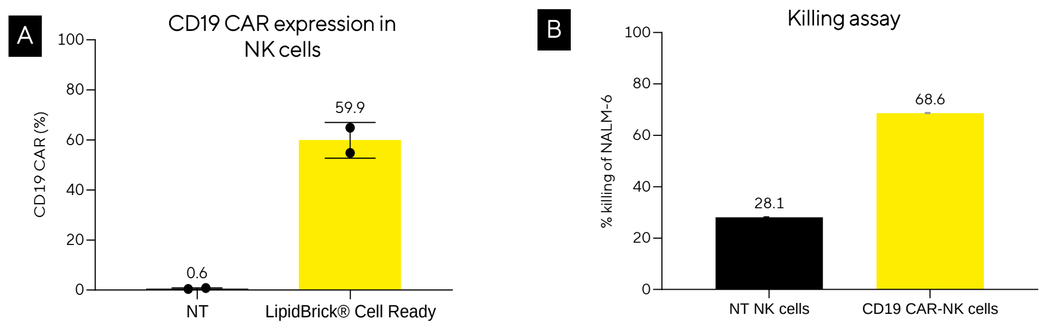

Figure 7: *(A) Expression of CD19 CAR was analyzed by flow cytometry one day after the transfection on NT and transfected NK cells with 0.80 µg mRNA/million cells (n=2 donors). (B) Fresh NT NK cells or transfected CD19 CAR-NK cells were co-cultivated with CellTrace Violet-labeled NALM-6 cells in 1:1 effector to target ratio. After 24 hours, killing of target cells by ex vivo mRNA based CD19 CAR-NK cells or NT NK cells was assessed by PI+ CellTrace Violet+ target cells.*

7. Conclusion

These results highlight the significant advantages of our innovative non-viral transfection solution LipidBrick® Cell Ready :

- Cost-Efficiency: ready-to-use reagent, no specific equipment nor consumables required.
- High performance: excels in transient expression & stable modification using gene editing.
- High cell viability: promotes optimal cell viability, growth, and functionality.
- Scalability: supports applications from well-plate to bioreactor production.
- Versatility: accommodates diverse nucleic acid payloads across various cell types.