Safer, Scalable T Cell Engineering

Minicircles as key starting materials to enable non-viral engineering of T lymphocytes for gene modified cell therapies

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Introduction

Lentiviral vectors (LVVs) are the current standard for CAR-T cell generation but remain costly, complex to manufacture, and carry safety risks like insertional mutagenesis. Virus-free gene transfer methods are attractive alternatives, yet conventional plasmids are limited by size, bacterial sequences, and poor expression. Minicircle DNA, being smaller, free of functional bacterial sequences, and supercoiled, overcomes these issues and enables more efficient, scalable, and safer development of engineered cellular therapies such as CAR-T, TCR-T and TIL. Current data show successful use in transposon systems, and minicircles may also serve as templates for future in vivo delivery via lipid nanoparticles.

Proprietary Minicircle Technology

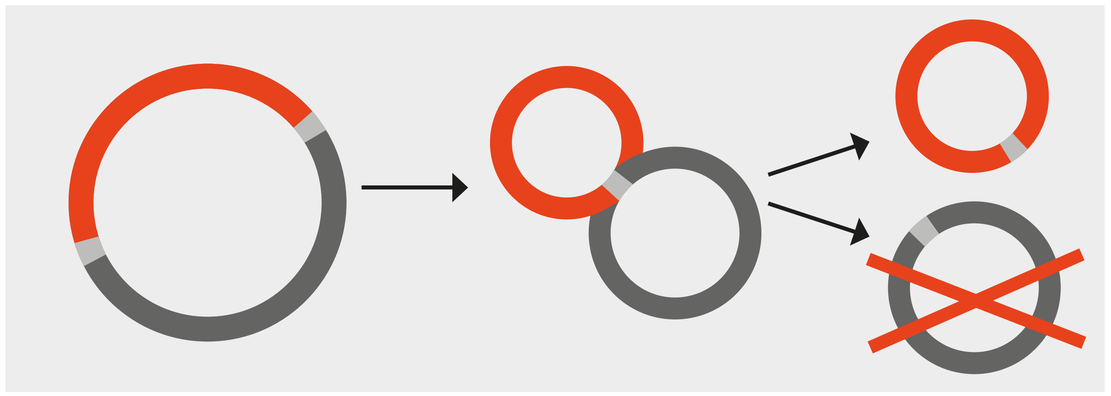
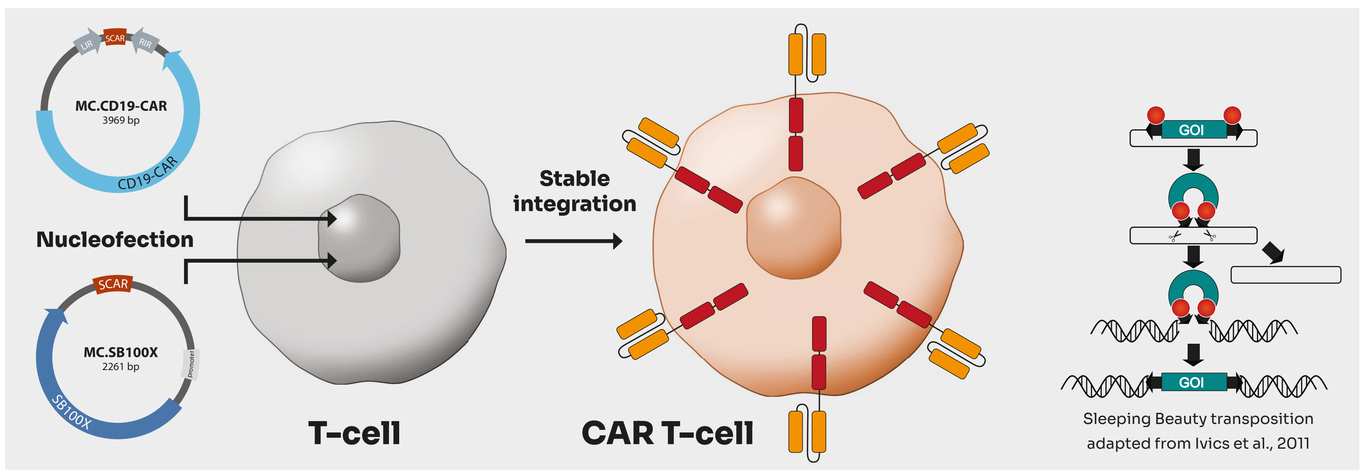


Fig. 1 – Minicircle design and functional advantages in cell engineering.
Minicircles are generated from parental plasmids by intramolecular recombination, removing functional bacterial sequences such as the origin of replication and antibiotic resistance genes.
The resulting small, supercoiled DNA carries only the therapeutic transgene cassette, enabling higher transfection efficiency, stronger expression, reduced toxicity, and improved safety compared to conventional plasmids.

✓ Higher yield ✓ Enhanced transfection ✓ Increased expression ✓ Less toxicity

Virus-free Generation of Engineered Cellular Therapies

Fig. 2 – Minicircles as efficient backbones for non-viral gene transfer.
Minicircle DNA enables virus-free engineering of e.g. CAR-T, TCR-T, TIL, and CAR-NK cells via Sleeping Beauty transposition. In co-transfection, the Minicircle carries the gene of interest and the transposase is provided either as Minicircle or mRNA. High-quality Minicircles support stable expression and efficient immune cell engineering with reduced toxicity compared to plasmids. GMP-grade Minicircles will be required for late-phase clinics and commercial use.



✓ Virus-free ✓ Scalable up to GMP ✓ Clinically validated ✓ Better safety

In vivo antitumor efficacy and safety

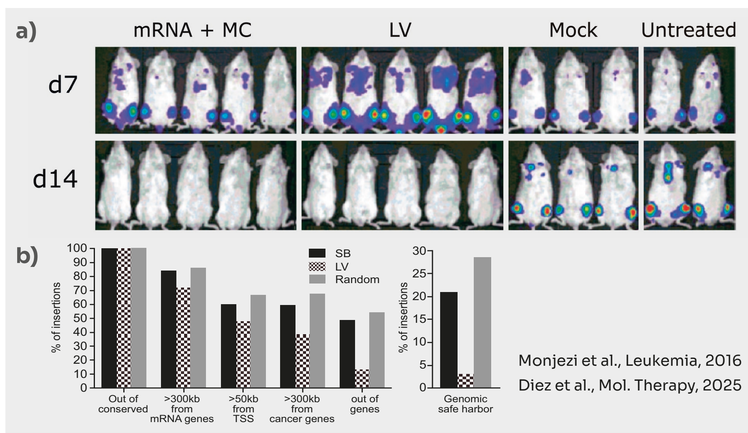
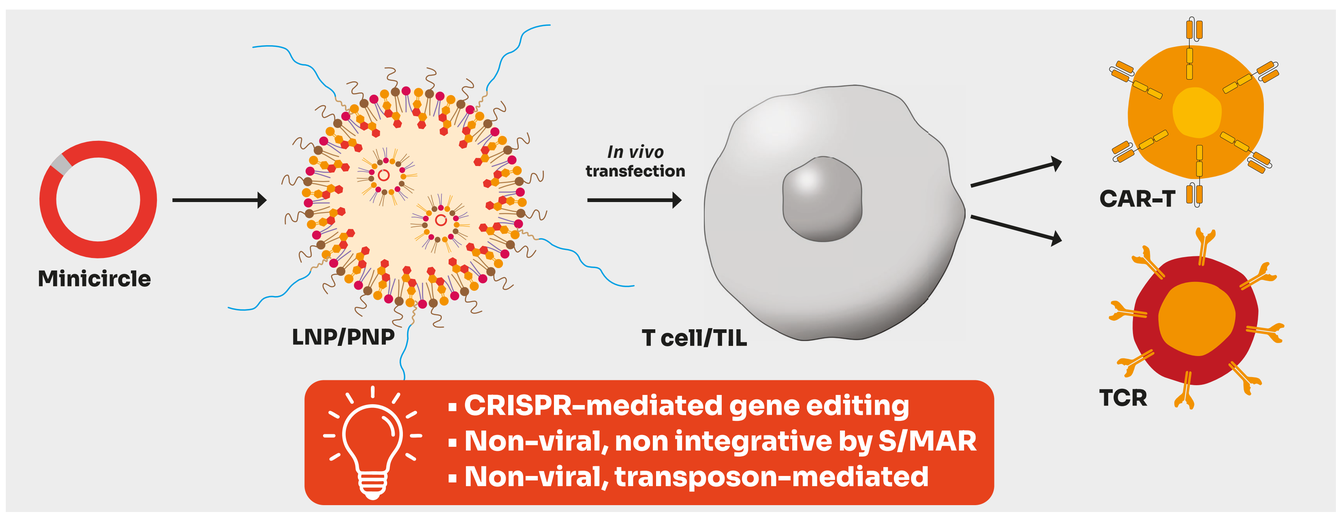


Fig. 3 – In vivo efficacy, safety, and clinical readiness of Minicircle-based CAR T cells.
(a) Bioluminescence imaging of Raji-ffLuc lymphoma in NSG mice shows CD19-CAR T cells generated with SB100X mRNA and Minicircle DNA rapidly eradicated tumors, while control mice developed progressive disease. (b) Insertion-site analysis shows SB-transposon CAR T cells from Minicircles integrate preferentially into genomic safe harbors (20.8% vs. 3% for LVV), reducing insertional mutagenesis risk. (c) PlasmidFactory's new GMP facility underlines readiness to provide Minicircle DNA for clinical and commercial translation.

✓ Efficacy as with LVV ✓ Stable integration & persistence ✓ Clinical applicability

Conclusion & Outlook



Clinically validated in multiple trials:
- CARAMBA
- TranspoCART19
- LION-1

Fig. 4 – Future outlook:
Minicircles for direct in vivo engineering of T cells and TILs.
Minicircles can be formulated with lipid or polymer nanoparticles (LNPs/PNPs) to enable systemic, non-viral immune cell engineering in vivo. This avoids the insertional risks of LVVs and may broaden engineered T cell therapy applications, including CAR-T, TCR-T, and TIL-based approaches for solid tumors.

✓ Compatible with LNP/PNP approaches ✓ Enables in vivo CAR-T & TIL therapies ✓ Avoids LVV risks

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