3101： Stable and efficient gene modification of tumor-infiltrating lymphocytes across multiple cancer types using non-viral electroporation-based NovaGMP platform

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Background

- TIL therapy has become a research hotspot in the field of solid tumor immunotherapy. To date, the first native TIL therapeutic product has been successfully commercialized. As the next-generation evolutionary direction of TIL therapy, genetically modified TILs have evolved into a core focus of industrial research and layout, with relevant clinical trials and pipeline research and development being continuously accelerated.
- Viral-based TIL gene modification faces safety concerns, high costs, and variable transduction efficiency.
- To overcome these challenges, we developed NovaGMP, a non-viral electroporation method specifically optimized for TILs. This platform delivers a piggyBac transposon-based plasmid (GC203) encoding a glycosylphosphatidylinositol (GPI)-anchored membrane bound IL-7 (mbIL-7-GPI).
- Our approach aims to enable efficient, stable, and consistent gene transfer across diverse cancer types.
- Here, we show that the NovaGMP platform enables stable, efficient, and reproducible gene modification of effector cells and TILs across multiple solid tumor types, with high transfection efficiency, robust secretion of cytotoxic cytokines, potent cytolytic activity, and stable cellular phenotype.
- In addition, NovaGMP platform exhibits markedly lower overall manufacturing costs than viral vector systems. It improves safety and accessibility while maintaining high gene modification efficiency.

Methods

- Cells samples
  - Effector cells derived from multiple donors.
  - TILs were isolated from patients with gynecological tumors (n=18), gastrointestinal tumors (n=15), melanoma (n=7), and other tumors (n=5).
- Gene editing
  - Cells were modified using the NovaGMP platform (non-viral electroporation with piggyBac transposon-based plasmid encoding target genes) (Figure 1).
- Effector cells editing assessments
  - Transfection efficiency (GFP$^+$ expression)
  - Immunophenotyping: CD3$^+$/CD4$^+$/CD8$^+$subsets
  - Functional outcomes: IFN-γ and TNF-α secretion, cytotoxicity (24-h killing assay)
- TILs editing assessments
  - Transfection efficiency (mbIL7$^+$ expression)
  - Immunophenotyping: CD3$^+$/CD4$^+$/CD8$^+$subsets,exhaustion markers (CD39, LAG-3, PD-1, CD57), TBNK populations

Results

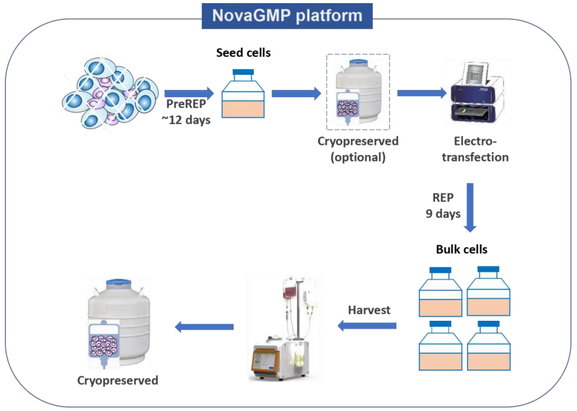
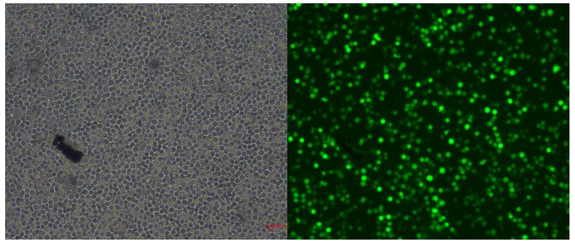


Figure 1. Schematic flowchart of the NovaGMP manufacturing process.
The NovaGMP (Non-Viral Gene-Modification Platform) platform employs a PiggyBac transposon system delivered via electroporation to enable high-efficiency, non-viral gene modification of TILs, offering a safer, cost-effective, and scalable approach to next-generation cellular immunotherapies.



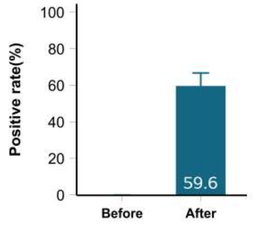


Figure 2. Transfection of effector cells by electroporation based on NovaGMP Platform.
Effector cells derived from multiple donors were transfected with the EGFP plasmid to evaluate the gene modification efficiency via the NovaGMP platform. After stable expression of the EGFP gene, remarkably strong GFP fluorescence signals were observed under fluorescence microscopy. GFP+ expression, indicating transfection efficiency, was 59.6% after gene stable integration ($p < 0.05$), demonstrating efficient gene transfer.

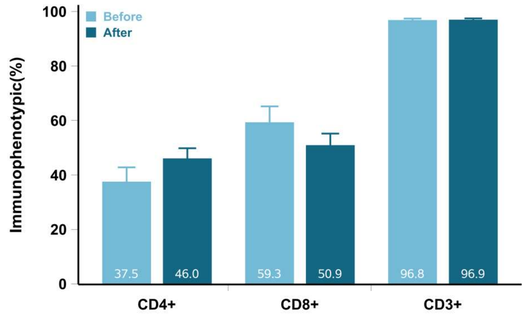


Figure 3.Immunophenotypic profiles of effector cells before and after electroporation using the NovaGMP platform.
Effector cells derived from multiple donors were transfected with the GC203 plasmid via the NovaGMP platform. After expansion, cells were stained for surface markers and analyzed by flow cytometry. Data represent mean percentages of CD3$^+$, CD8$^+$, CD4$^+$, subsets within the viable lymphocyte gate. The overall immunophenotypic profiles remained within ranges considered not clinically significant before and after NovaGMP-mediated gene transfer. No functional impairment associated with these variations was detected (see IFN-γ, TNF-α and cytotoxicity data).

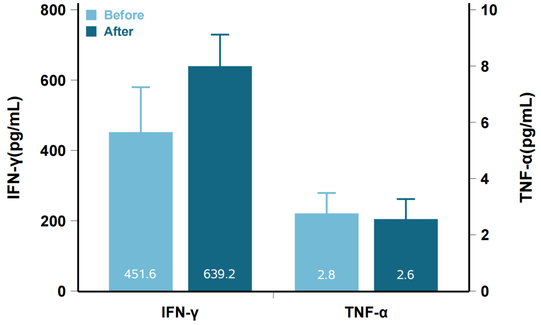


Figure 4. IFN-γ and TNF-α secretion by effector cells before and after NovaGMP-mediated gene transfer.
Effector cells isolated from multiple donors were transfected with the GC203 plasmid via the NovaGMP platform. Under stimulated conditions, IFN-γ and TNF-α secretion were observed. IFN-γ production was markedly elevated after electroporation ($p < 0.05$), while TNF-α production exhibited no statistically significant differences before and after electroporation ($p > 0.05$). These results confirm that the NovaGMP platform preserves the functional capacity of engineered effector cells to secrete IFN-γ and TNF-α in response to activation signals, and GC203 plasmid effectively promoted the secretion of IFN-γ.

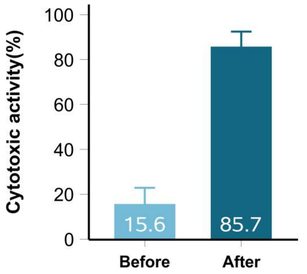


Figure 5. Cytotoxic activity of effector cells before and after NovaGMP-mediated gene transfer in a killing assay.
Effector cells isolated from multiple donors were electroporated with GC203 via the NovaGMP platform and tested in a cytotoxicity assay. The percentage of target cell lysis is shown for effector cells before and after electroporation. Cytotoxic activity was significantly enhanced after electroporation ($p < 0.05$) , indicating that the non-viral gene editing process effectively potentiated the cytolytic function of the effector cells.

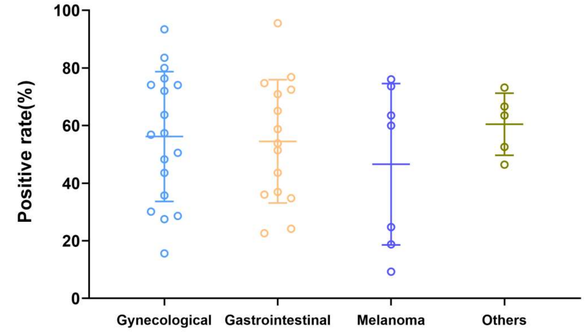


Figure 6. Transfection efficiency evaluated by mbIL7+ expression in TILs from different tumor types after NovaGMP-mediated gene transfer.
TILs isolated from different tumor types (gynecological, gastrointestinal,melanoma,and others) were transfected with the GC203 plasmid via the NovaGMP platform. And mbIL7+ expression, indicating transfection efficiency, was similar across all groups, with no significant intergroup differences ($p > 0.05$), demonstrating consistent gene transfer. Furthermore, immunophenotypic profiles (CD3$^+$, CD8$^+$, CD4$^+$, TBNK subsets) and exhaustion markers (CD39, LAG-3, PD-1, CD57) showed no clinically significant variation.

Conclusions

The NovaGMP platform enables stable, efficient, and reproducible gene modification of effector cells and TILs across diverse cancer types, with high transfection efficiency, robust secretion of cytotoxic cytokines, potent cytolytic activity, and stable cellular phenotype. This non-viral approach may offer a more cost-effective strategy for TIL engineering, and its consistent performance across tumor types could facilitate broader clinical exploration in adoptive cell therapies.