Enabling Translational Immunotherapy Through *Non-Viral Delivery to Immune Cells*

Aarushi Jain* [1], Zhao Han [1], Rajasekhar Ponduri [1], Haritha Menon [1], Maria Albaba [1], Athaliah Gervacio [1], Jennifer Nguyen [1], Yury Karpov [1], Jun Liu [1], Rajesh Krishnan [1], Natalia Martin-Orozco [1]
(1) Providence Therapeutics Holdings Inc., Calgary, AB, CANADA

PLATFORM
Lipid-Based / Self-assembly LNP

PAYLOAD TYPES
mRNA · Gene-Editing Cargos

CELL SYSTEMS
PBMCs · Jurkat · KHYG-1

APPLICATION
Preclinical Immunotherapy R&D

01 BACKGROUND

The Challenge of Immune Cell Transfection

Efficient genetic manipulation of immune cells is central to translational immunotherapy, enabling applications in immune modulation, gene editing, and engineered cell therapeutics.

Non-viral delivery in primary immune cells, particularly PBMCs — remains challenging due to:

VARIABLE EFFICIENCY
Inconsistent payload delivery across experiments and donors

CYTOTOXICITY
Cell health compromised by existing transfection methods impairs downstream manufacturing yield

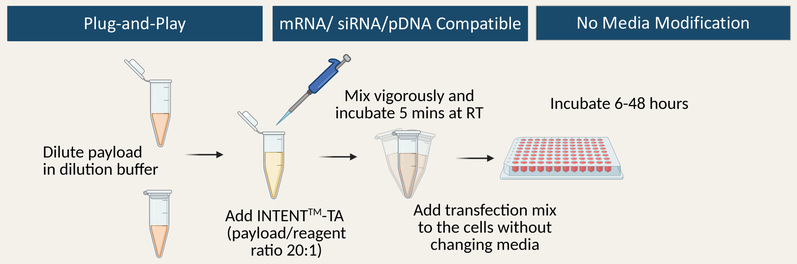
LIMITED REPRODUCIBILITY
Constrains *in vitro* research and translational development

PLATFORM DESIGN

DoE-Guided Discovery Framework

A lipid-based, non-viral delivery system was developed using a Design of Experiments (DoE) approach to systematically identify optimal candidate. INTENT™-TA is a self-assembling lipid nanoparticle transfection agent.

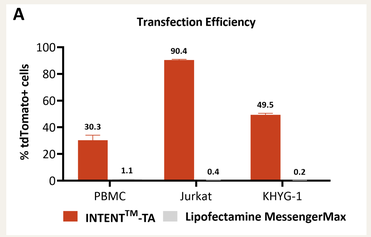
Designed to maximize intracellular payload delivery while **preserving immune cell health** — no specialized instrumentation required.



02 KEY RESULTS

Performance Across Cell Systems

Evaluated across diverse immune cell models including human PBMCs, Jurkat T cells, and KHYG-1 cells using a flow cytometer



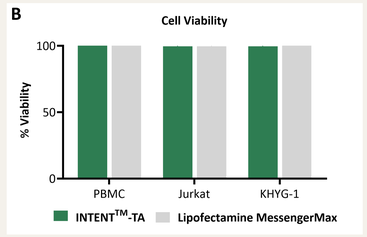


Figure 1. INTENT™-TA transfects cells with minimal toxicity and high efficiency.
huPBMCs (gated on monocytes), Jurkat and KHYG-1 cells in a 96-well plate were transfected with tdTomato mRNA using INTENT™-TA or Lipofectamine MessengerMAX at 242 ng/well. Transfection efficiency (A) and Viability (B) were analysed by flow cytometry 24 hours post-transfection.

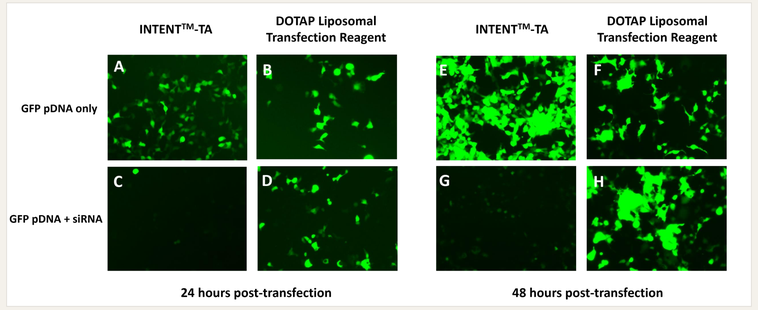


Figure 2. siRNA-mediated knockdown of GFP expression following co-delivery with pDNA in HEK293 cells.
(A–D) 24h and (E–H) 48h post-transfection using INTENT™-TA (A,C,E,G) or DOTAP Liposomal Transfection Reagent (B,D,F,H). Top row: GFP pDNA only; bottom row: GFP pDNA + siRNA. Reduced GFP fluorescence in co-delivery condition (C,D,G,H) confirms successful siRNA knockdown.

KEY FINDINGS

>90% transfection efficiency in Jurkat T cells, **>30%** in huPBMCs and **>49%** in KHYG-1 NK cells— outperforming commercial reagent (Fig. 1A)

>98% viability maintained across all immune cell types post-transfection (Fig. 1B)

Simultaneous siRNA knockdown and transgene expression confirmed in a single step— demonstrating multi-payload co-delivery (Fig. 2)

03 SIGNIFICANCE

Scalable Platform, Broad Applicability

Improved performance was achieved without cell-type–specific optimization, supporting ease of implementation and experimental reproducibility.

1. **NO SPECIALIZED EQUIPMENT**
Standard laboratory conditions; no modifications to culture media or serum

2. **MULTI-PAYLOAD COMPATIBILITY**
Supports mRNA and gene-editing cargos via rapid plug-and-play preparation

3. **CROSS–CELL TYPE PERFORMANCE**
Consistent results across PBMCs, Jurkat, and KHYG-1 without re-optimization

4. **TRANSLATIONAL POTENTIAL**
Robust performance establishing strong preclinical foundation; in vivo validation underway

5. **STABLE AND SCALABLE**
Maintains performance after storage and handling at room temperature for up to 6 months

CONCLUSION

INTENT™-TA delivers **high transfection efficiency** with **>98% viability** across clinically relevant immune cell types — without viral vectors, media changes, or specialized equipment. Its easy **5-minute self-assembling LNP protocol** and **multi-payload compatibility** establish it as a highly effective, **user-friendly transfection reagent** for both **cell lines and primary immune cells-** paving foundation in Canada's growing CGT ecosystem.

CONTACT

Business Development: bd@providencetherapeutics.com
Inquiries: transfectionagent@providencetherapeutics.com

Non-Viral Gene Delivery Platform · Immune Cell Engineering · Translational Immunotherapy · Lipid-Based · DoE-Optimized · mRNA + Gene-Editing Compatible · BioCanRx Summit4CI

Non-Viral Delivery | PBMCs | mRNA | Gene Editing