Nanoparticle Mediated Non-Viral Delivery of Messenger RNA in Human T Cells Towards Development of CAR T-Cell Therapy

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Introduction

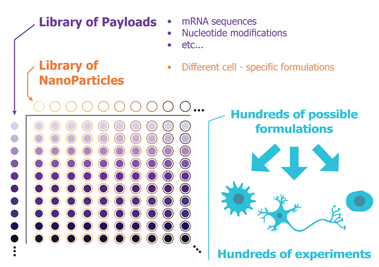
- For CAR T-cell therapies and other cell therapies to meet clinical demand, safer, scalable alternatives to viral vectors are needed to reduce cell processing and manufacturing times
- Non-viral methods such as electroporation avoid these safety concerns but require a trade-off in performance between efficiency and cell viability
- PNI's new hybrid Lipid Nanoparticle technology provides high performance in sensitive and difficult to transfect cells.
- NanoAssemblr® production allows rapid, reproducible encapsulation from µL to L scale
- Performance and manufacturing advantages of hybrid LNPs were demonstrated here by optimizing mRNA delivery to primary human T cells

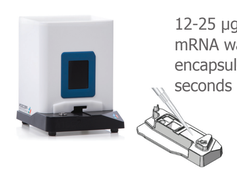
Methods Overview: An Extensive and Diverse Nanoparticle Library Maximizes Chances of a Hit

Gene Delivery via hybrid Lipid Nanoparticles

- Precision NanoSystems' proprietary hybrid lipid nanoparticle (LNP) technology encapsulates nucleic acids and delivers them into the cytoplasm of cells using natural endocytic pathways
- The advantages of PNI's hybrid LNPs include:
  - High transfection efficiencies
  - No observable impact on cell physiology
  - Nucleic acids are protected from degradation to maintain potency
  - Simple 1-step administration to cells amenable to all culture workflows
- Fast, simple and scalable manufacturing using the NanoAssemblr® platform
- Nanoparticles are rapidly optimized for new cell types, payloads and culture conditions using the NanoAssemblr® Spark in vitro workflow (Right)

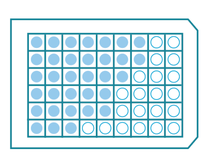
Rapidly Screen Nanoparticle or RNA Library with NanoAssemblr® Spark™



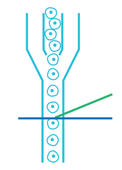


12-25 µg of mRNA was encapsulated in seconds

Reporter gene encapsulated in 26 different hybrid LNP formulations



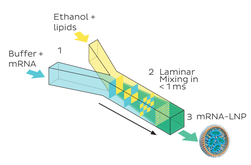
Formulations were immediately applied directly to primary human T cells



Formulations and conditions were screened for transgene expression

Readily Scale Lead Formulations

The NanoAssemblr platform uses microfluidics to precisely control the manufacturing of nanoparticles from microlitre to litre scales.

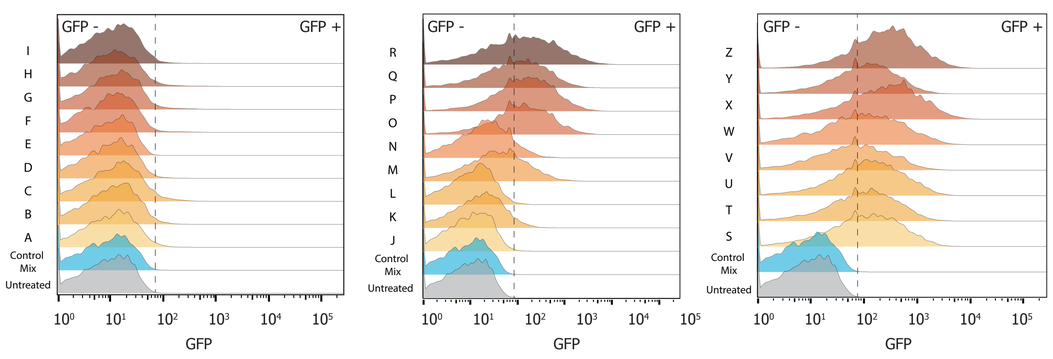


NanoAssemblr Systems Span All Scales

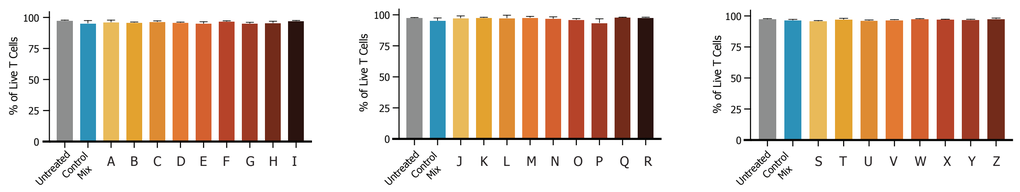
Demonstration of Gentle, yet Potent Gene Delivery for Ex Vivo Human T Cell Applications

1. A Diverse Field of Candidate Formulations Was Screened in Primary Human T Cells and Several High Performance Leads Were Selected

A. Flow Cytometry Shows Some Formulations Result In High GFP Expression Across Large Fraction of Cell Population

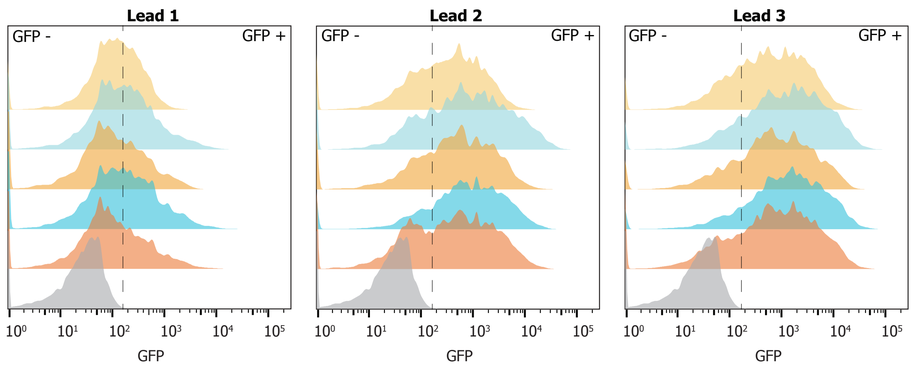


B. Cell Viability Is Unaffected By Hybrid LNP Treatment And Expression Of The Transgene, Important for Achieving A High Yield Of Modified Cells



2. Lead Hybrid LNPs Were Versatile in Mediating Transgene Expression in Multiple T Cell Subpopulations

A. Flow Cytometry Indicates Each Hybrid LNP Performs Consistently Across All T Cell Subtypes



	T Cell Isolation	Gating
	CD8	Live
	CD4	Live
	Pan T	Live + CD8+
	Pan T	Live + CD4+
	Pan T	Live
	Untreated, Pan T	Live

▲ Further Details

Human T cells were isolated using pan T-cell markers or CD4 or CD8, then treated wtih hybrid LNPs and stained with a viability stain, FVS 570 (BD Biosciences) and analyzed by flow cytometry. Populations were gated for live T cells only or CD4+ or CD8+ and GFP fluorecence was quantified. Histograms are representative replicates from duplicate samples.

Treatment Details

Cell Type	primary human T cells from whole blood
Payload	GFP mRNA
Treatment Point	post activation
Dose	2 µg/500K cells
Apo-E	1 µg/mL media
Treatment time	48 Hours

◀ Further Details

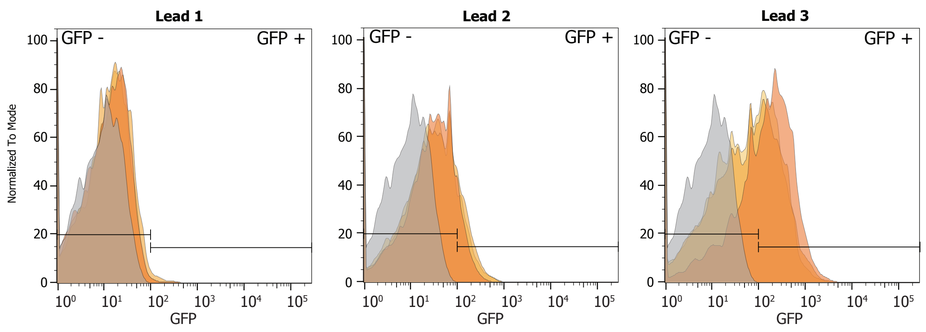
Human T cells were isolated using pan T-cell markers and treated wtih hybrid LNPs then stained with a viability stain, FVS 570 (BD Biosciences) and analyzed by flow cytometry. Cells were were gated for only live cells and GFP fluorecence was quantified (A). Histograms are representative replicates from duplicate samples. Live cells were quantified and expressed as a percentage of the population (B).

Treatment Details

Cell Type	primary human T cells from whole blood
Payload	GFP mRNA
Treatment Point	post activation
Dose	2 µg/500K cells
Apo-E	1 µg/mL media
Treatment time	48 Hours

3. Hybrid LNPs Allow Treatment Before, After, or Simultaneously with T-Cell Activation, Enabling Flexible Incorporation Into Workflow

Flow Cytometry Measuring GFP expression in Human T-Cells Indicates Formulation 3 Is Most Robust to Different Treatment Timing Relative To T Cell Activation



	Treatment & Activation
	Untreated
	Treat 6H then Activate
	Activate 6H then Treat
	Activate & Treat

Treatment Details

Cell Type	primary human T cells from whole blood
Payload	GFP mRNA
Dose	2 µg/500K cells
Apo-E	1 µg/mL media
Treatment time	48 Hours

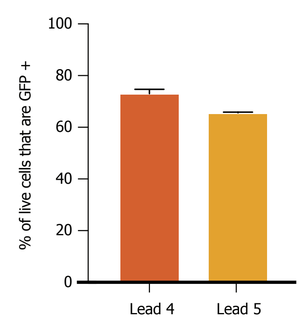
Further Details

Human T cells were isolated using pan T-cell markers and treated wtih hybrid LNPs then stained with a viability stain, FVS 570 (BD Biosciences) and analyzed by flow cytometry. Cells were were gated for only live T cells and GFP fluorecence was quantified. Histograms are representative replicates from duplicate samples.

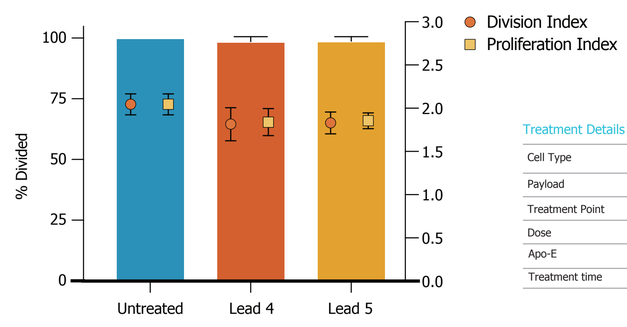
4. T Cell Proliferation Is Not Affected Following Hybrid LNP-Mediated Transfection

T Cells were tracked for 4 days following Hybrid LNP Transfection

A. High GFP Expression Levels Maintained After 96h



B. Flow Cytometry-based Proliferation Analysis Shows Cell Division Is The Same Between Treated And Untreated Controls



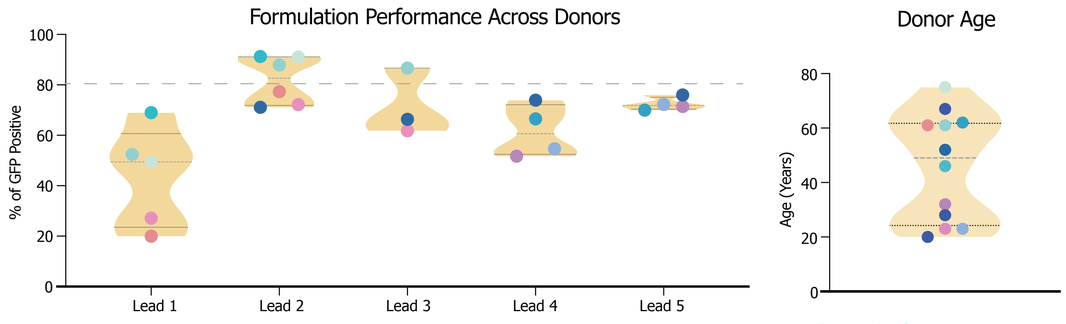
Treatment Details

Cell Type	primary human T cells from whole blood
Payload	GFP mRNA
Treatment Point	post activation
Dose	2 µg/500K cells
Apo-E	1 µg/mL media
Treatment time	96 Hours

Further Details

Human T cells were treated with proliferation dye followed by nanoparticles, and viability dye and analyzed by flow cytometry. Proliferation analysis was conducted using the the proliferation module in the FlowJo software. Data points represent the average of two replicates, and error bars represent standard deviation

5. Hybrid LNPs Induce Consistent and High Transgene Expression Across A Range of Ages in Both Sexes



Further Details

Flow cytometery following treatment with hybrid LNPs. Each point represents the average of two replicates. The color of each point remains the same for each individual donors in both graphs. The blue/green toned symbols represent male donors and the pink/purple tones represent female donors.

Treatment Details

Cell Type	primary human T cells from whole blood
Payload	GFP mRNA
Treatment Point	post activation
Dose	2ug/500K cells
Apo-E	1 µg/mL media
Treatment time	48 Hours

Conclusions

- Performance and manufacturing advantages of hybrid LNPs were demonstrated by delivering mRNA to primary human T cells
- The NanoAssemblr Spark workflow allowed rapid, efficient screening of a proprietary nanoparticle library
- Several lead nanoparticles exhibited transfection efficiencies and viability exceeding 80% and 95%, respectively
- In depth studies demonstrated robust performance of hybrid LNPs across different T cell subtypes, activation stages, donor age and sex
- Health and proliferation of transfected cells were unperturbed following hybrid LNP treatment.
- Studies to evaluate the delivery of CAR and other functional constructs to T cells using these lead nanoparticles are currently underway.
- These results highlight the utility of LNP technologies to aid in the development of next-generation CAR T therapies and other cell-based therapies

Detailed Methods

T cell isolation
Pan T cell isolations were performed as per manufacture's instructions from human whole blood using the EasySep™ Direct Human T Cell Isolation Kit (StemCell Cat. #19611). CD4+ and CD8+ T cell isolations were also performed as per manufacture's instructions from human whole blood using the EasySep™ Direct Human CD4+ T Cell Isolation Kit (StemCell Cat. #19662) and EasySep™ Direct Human CD8+ T Cell Isolation Kit (StemCell Cat. #19663). Cells were maintained in ImmunoCult-XF T cell Expansion medium (StemCell Cat. #10981) supplemented with Recombinant Human IL2 (Peperotech, Cat# 200-02). Isolated T cells were activated at the time of isolation (unless otherwise specified) using a CD3/CD28/CD2 activator (StemCell. Cat # 10970).

Nanoparticle manufacturing
Nanoparticles were formulated using the NanoAssemblr Spark instrument (PNI Cat # NIB0010) and accompanying microfluidics cartridge (PNI Cat # NIS0009). The encapsulated mRNA encodes eGFP with a CleanCap modification (Trilink, Cat# L-7601)

T Cell treatment with nanoparticles
T cells were treated several days following activation unless otherwise specified. T cells were treated at a dose of 2ug of mRNA per mL of media in the presence of 1ug/mL of ApoE (Peperotech, Cat# 35004). Cells were left in the presence of the nanoparticles until cells were harvested for flow cytometry.

Flow cytometer analysis
Following 48 hours of treatment with the nanoparticles, the cells were harvested for flow cytometry analysis. In short, cells were washed with PBS prior to staining with FVS 507 (BD Cat # 564995), after staining cells were then washed with Stain Buffer BSA (BD, cat# 554657). The cells were then stained with the following antibodies, CD25 (BD, cat# 560503), CD4 (BD, cat# 566319), and CD8 (BD, cat # 563824). Prepared samples were then run on a BD FACSCelesta flow cytometer (BD). Data was analysed using FlowJo Software Version 10.

Proliferation Assay
Prior to treatment cells were pulsed with V450 Proliferation Dye (BD Cat # 562158). 4 days following pulse and treatment, cells were harvested for flow cytometery. All analysis were completed on live cells only. Proliferation modeling was completed using the Proliferation Module of the FlowJo Software.