WHITE PAPER | Cell Engineering Solutions

A New Paradigm in Viral Transduction

Transient Fusion-Promoting Peptides for Enhanced Cell and Gene Therapy Manufacturing

In this white paper we discuss:

- The fundamental limitations of current viral transduction approaches and the critical unmet need in cell therapy manufacturing
- The HiTE™ Transient Fusion-Promoting Peptide (TFP) technology platform and its mechanism of action
- Performance data across clinically relevant cell types: CD3+ T cells, iPSCs, and NK cells
- Safety, workflow, and cost-impact advantages for GMP manufacturing
- Application use cases spanning CAR-T, CAR-NK, iPSC-derived, and gene therapy programs

Introduction

Viral transduction is the cornerstone of modern cell and gene therapy manufacturing. From chimeric antigen receptor T (CAR-T) cell production to iPSC-derived allogeneic platforms and hematopoietic stem cell (HSC) gene correction, efficient genetic modification of primary immune and stem cells is central to realizing the clinical potential of next-generation immunotherapies.

Yet despite decades of optimization, a fundamental bottleneck remains—primary immune cells, particularly T cells, natural killer (NK) cells, and pluripotent stem cells—are inherently resistant to viral transduction. Conventional transduction enhancers either compromise cell health, introduce genotoxic risk, or impose prohibitive manufacturing complexity that impedes scale-up and clinical adoption.

HiTE™ (High-Efficiency Transduction Enhancer) represents a new class of synthetic biomolecules—Transient Fusion-Promoting Peptides (TFPs)—engineered to address these limitations at their root. By transiently enhancing viral vector entry through a controlled, self-inactivating mechanism, HiTE™ achieves superior transduction efficiency across the most challenging primary cell types without sacrificing viability, function, or safety. Here we describe the HiTE™ technology platform, its performance across key cell types, and its potential to reshape the economics and accessibility of cell therapy manufacturing.

The Challenge: Current State of Viral Transduction

The Transduction Bottleneck

Efficient genetic modification of primary immune and stem cells remains one of the most consequential technical challenges in gene therapy and immunotherapy. The clinical promise of CAR-T cell therapy, for example, depends on reliably introducing a therapeutic transgene into patient-derived T cells at high efficiency and low toxicity. Despite years of protocol optimization, current viral transduction methods face fundamental limitations that affect both research reproducibility and commercial manufacturing at scale.

Primary T cells, NK cells, and iPSCs present a uniquely hostile environment for viral vector entry. Rigid plasma membranes, low surface receptor expression, and endogenous antiviral mechanisms collectively suppress transduction rates, requiring high multiplicity of infection (MOI) to achieve acceptable transgene delivery. This in turn drives up viral vector consumption—which represents the single largest cost driver in cell therapy manufacturing—while simultaneously elevating genotoxic risk through elevated vector copy number (VCN).

The Unmet Need

The field currently lacks a solution that simultaneously addresses efficiency, safety, and manufacturability. An ideal transduction enhancer must achieve high transduction efficiency (>50%) at low MOI, maintain cell viability above 90%, eliminate complex and time-consuming workflows, integrate with automated GMP platforms, and minimize genotoxicity risk through controlled VCN.

HiTE™ Technology Platform: Transient Fusion-Promoting Peptides

HiTE™ represents a new class of synthetic peptides—Transient Fusion-Promoting Peptides (TFPs)—engineered to transiently enhance viral vector entry into cells that are inherently resistant to transduction. These modular constructs integrate multiple functional domains to achieve controlled membrane destabilization during a strictly limited temporal window, followed by self-inactivation that preserves downstream cell health and function.

Unlike broad-spectrum membrane-disrupting agents such as Polybrene, TFPs are designed with precision specificity. Their activity is temporally constrained: after facilitating viral entry, the peptide undergoes self-inactivation, removing the membrane-destabilizing stimulus before it can compromise cell integrity or perturb downstream signaling. The result is a transduction enhancer that acts decisively when needed and disappears before causing harm—a distinction with profound implications for both efficacy and safety.

HiTE™ represents a fundamental advancement in viral transduction technology. By combining targeted membrane fusion enhancement with built-in self-inactivation mechanisms, HiTE™ achieves what no existing enhancer can: superior efficiency with preserved cell viability and function.

Performance Data

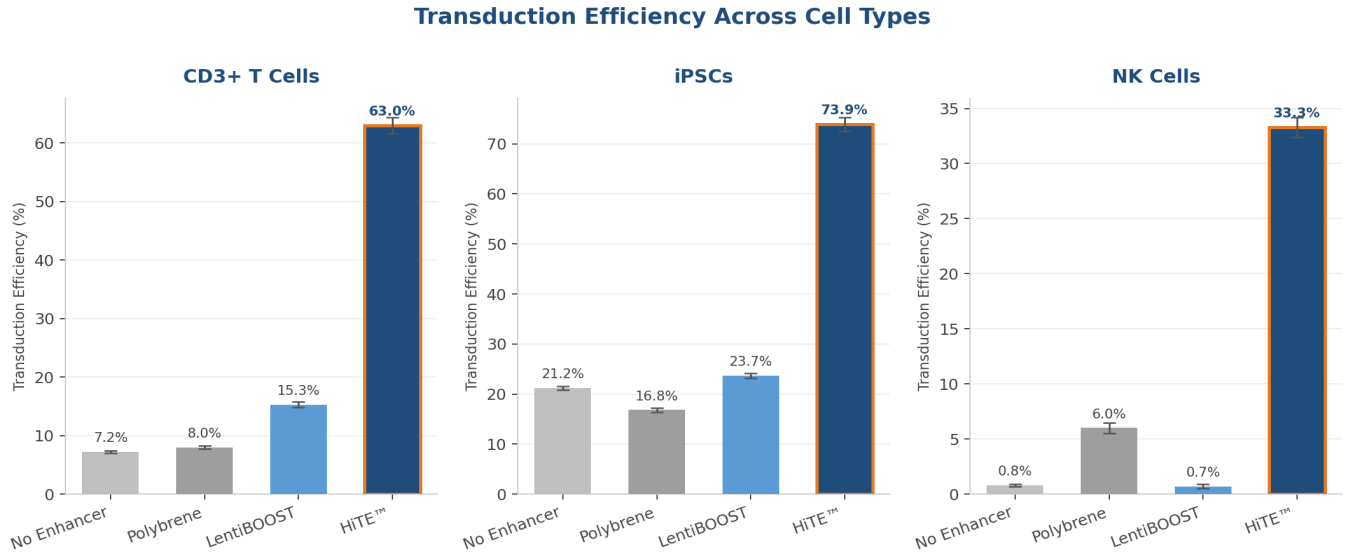

Figure 1. Transduction efficiency (% CD19-CAR-mCherry+) across primary CD3+ T cells, iPSCs, and NK cells for each enhancer condition. All data presented were generated using CD19-CAR lentiviral constructs at Day 3 post-transduction, with n=3 independent biological replicates. *HiTE™ demonstrates statistically superior performance in all three cell types (****p<0.0001).*

Primary Human CD3+ T Cells

Primary human T cells represent the most clinically relevant cell type for CAR-T therapy manufacturing. HiTE™ demonstrates exceptional performance, achieving 63.0% transduction efficiency—4-8× higher than the leading competitors.

Induced Pluripotent Stem Cells (iPSCs)

iPSCs present unique transduction challenges due to their sensitivity to membrane-disrupting agents and the stringent requirement to preserve pluripotency throughout the transduction process. Loss of OCT4, NANOG, or SOX2 expression signals pluripotency compromise that renders the cell product non-viable for downstream differentiation workflows. HiTE™ achieves 73.9% transduction efficiency in iPSCs—3.1× higher than LentiBOOST.

Natural Killer (NK) Cells

NK cells represent one of the most formidable transduction challenges in cell therapy. Rigid plasma membranes, minimal receptor expression for common viral entry receptors, and extreme sensitivity to cationic polymers such as Polybrene—which induces greater than 80% viability loss—have historically rendered efficient NK cell transduction elusive. HiTE™ achieves 33.3% transduction efficiency at low MOI, representing a 47.6-fold improvement over the best-performing alternative (excluding the unusable Polybrene condition) while maintaining cell viability within normal parameters.

IMPORTANT: Polybrene Cytotoxicity in NK Cells

Polybrene (hexadimethrine bromide) causes severe cytotoxicity in primary NK cells, reducing viability to approximately 17% compared to a >80% baseline. This catastrophic cell death eliminates any transduction benefit and renders Polybrene clinically unsuitable for NK cell applications. HiTE™ maintains NK cell viability within normal parameters while delivering superior transduction rates.

Cell Viability

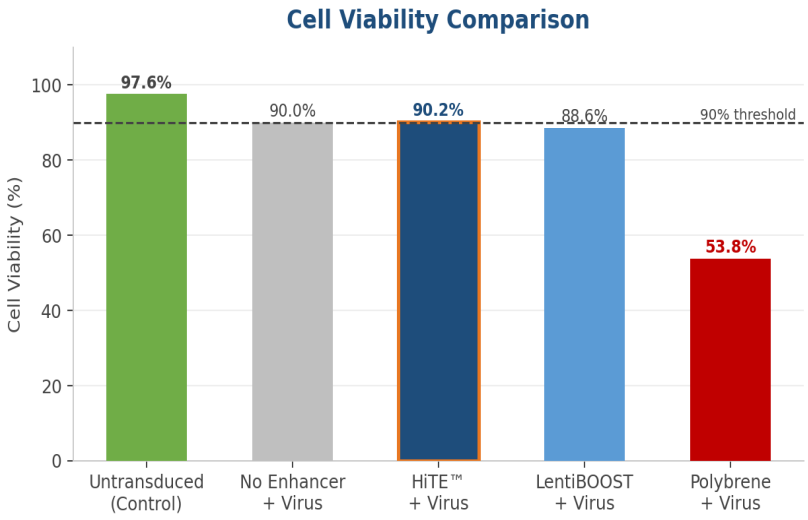

***Figure 2. Cell viability (%) by condition at Day 3, measured by flow cytometry viability dye staining**. HiTE™ viability is statistically equivalent to untransduced controls. p<0.05 HiTE™ vs. Polybrene.*

Transduction efficiency is clinically meaningful only when achieved with acceptable cell health. HiTE™ maintains greater than 90% cell viability, statistically equivalent to untransduced controls and far superior to Polybrene, which drives viability below 55%. This viability profile is consistent across donors and cell types, supporting reliable lot release for clinical manufacturing.

Condition	Viability
Untransduced	97.6%
No Enh. + Virus	90.0%
HiTE™ + Virus	**90.2%**
LentiBOOST + Virus	88.6%
Polybrene + Virus	53.8%

***Table 1. Cell viability at Day 3 by flow cytometry viability dye staining**.*

Safety Profile

Vector Copy Number (VCN)

Vector copy number (VCN) is a critical safety parameter in cell therapy manufacturing. Insertional mutagenesis risk scales with VCN, and regulatory guidance from the FDA and EMA recommends maintaining VCN below 5 copies per cell for clinical-grade gene-modified cell products. Achieving high transduction efficiency at high MOI often drives VCN above this threshold—representing a fundamental tension in current manufacturing approaches.

HiTE™ resolves this dilemma. By achieving high efficiency at low MOI, HiTE™ produces a mean VCN of 2.8 ± 0.1 copies per cell—well within the FDA-recommended safety threshold. In contrast, Polybrene at the same MOI yields a mean VCN of 7.0 ± 0.3, exceeding the threshold by 40% and introducing unacceptable genotoxic risk for clinical use.

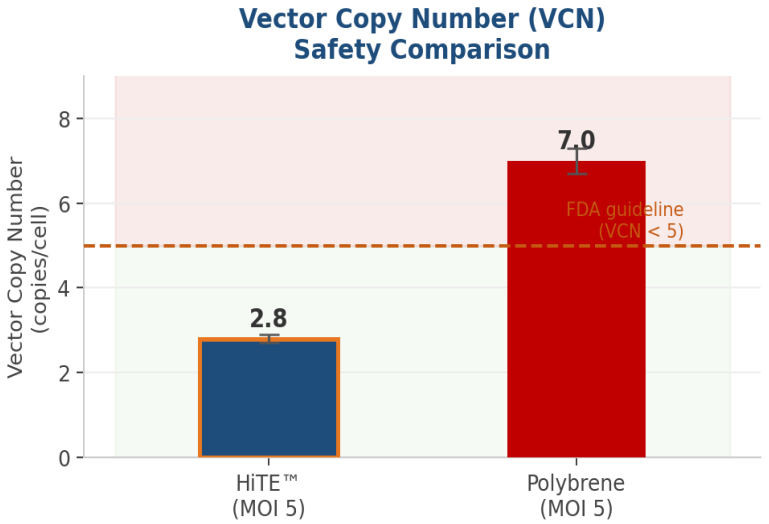

***Figure 3. Vector copy number (VCN) by qPCR quantification. n=3 independent T cell donors.** HiTE™ maintains VCN below the FDA-recommended threshold of 5 copies/cell. *p<0.05 vs. Polybrene.*

Condition	VCN (copies/cell)	Safety Assessment
HiTE™ (MOI 5)	**2.8 ± 0.1**	**✓ Below FDA threshold**
Polybrene (MOI 5)	7.0 ± 0.3	⚠ Exceeds FDA threshold

***Table 2.** VCN safety comparison. qPCR, n=3 donors. *p<0.05.*

Comparative Analysis

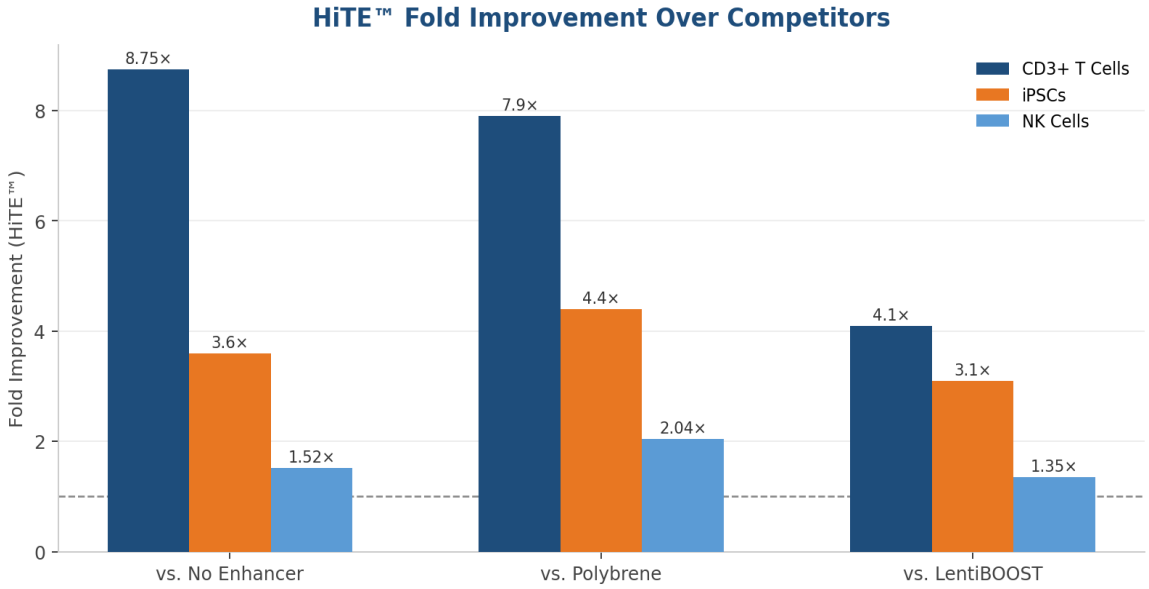

Figure 4. Fold improvement of HiTE™ over each comparator across CD3+ T cells, iPSCs, and NK cells. *All comparisons statistically significant (****p<0.0001 unless noted).*

HiTE™ Fold Improvement Over Competitors

Across all three primary cell types evaluated, HiTE™ consistently outperforms available transduction enhancers by substantial margins. The fold improvements documented above reflect reproducible, statistically significant differences observed across independent biological replicates and donor samples.

Head-to-Head Platform Comparison

Beyond transduction efficiency and viability, HiTE™ offers a comprehensive set of manufacturing advantages that differentiate it from all available alternatives across workflow simplicity, automation compatibility, and safety profile.

Parameter	HiTE™	Retronectin	Polybrene	LentiBOOST
T Cell Efficiency	63.0%	~15%	8.0%	15.3%
iPSC Efficiency	73.9%	~20%	16.8%	23.7%
NK Cell Efficiency	33.3%	Limited	6.0%*	0.7%
Cell Viability	>90%	70–80%	53.8%	88.6%
Workflow Time	<1 hour	>24 hours	<1 hour	<1 hour
Spinoculation Required	No	Yes	Optional	Optional
Plate Coating Required	No	Yes	No	No
Self-Inactivating	Yes	No	No	No
Automation Compatible	Yes	No	Partial	Yes

Table 3. Head-to-head platform comparison.

Manufacturing and Workflow Benefits

Simplified Protocol

Current gold-standard transduction protocols using retronectin require upwards of 24 hours of multi-step, open-system handling, introducing both process complexity and sterility risk. HiTE™ eliminates these barriers entirely, reducing the transduction workflow to less than two hours of closed-system processing with no plate coating, no spinoculation, and no overnight incubation.

Traditional Protocol (Retronectin)	HiTE™ Protocol
1. Coat plates with retronectin (2–4 h or overnight)	1. Add cells to standard plates
2. Block with BSA (30min)	2. Add HiTE™ + viral vector
3. Load virus (30 min–2 h)	3. Mix gently
4. Add cells to coated plates	4. Incubate 8 hours
5. Spinoculation (1,000×g, 90 min)	5. Optional: media change
6. Incubate overnight	
7. Media change	
Total: 24+ hours | Open-system handling	Total: <8 hours | Closed-system compatible

Table 4. Workflow comparison between traditional retronectin-based spinoculation and the HiTE™ protocol. *HiTE™ eliminates coating, spinoculation, and overnight incubation steps.*

Cost Impact Analysis

Viral vector production represents the dominant cost driver in cell therapy manufacturing, often accounting for 50–70% of total cost of goods (COGS). By enabling equivalent or superior transduction at 5 to 10 times lower MOI, HiTE™ directly reduces viral vector consumption per dose—with downstream implications for overall COGS and, ultimately, patient access.

Automation Compatibility

HiTE™ integrates seamlessly with closed automated manufacturing systems, including the Miltenyi CliniMACS Prodigy and Lonza Cocoon platforms, through direct addition to tubing sets with no manual intervention. This compatibility eliminates a key barrier to GMP-compliant scale-up. Internal benchmarking demonstrates a 2.17-fold increase in successful manufacturing run yield and a 92% reduction in total workflow time compared to 24-hour traditional protocols.

Applications

CAR-T Cell Manufacturing

The primary application for HiTE™ is ex vivo manufacturing of chimeric antigen receptor T cells. HiTE™ enables higher transduction rates with lower viral vector input, preserved T cell fitness, activation, and proliferation, low VCN for enhanced safety profile, and automation-compatible manufacturing.

CAR-NK Cell Therapy

NK cells represent an emerging allogeneic cell therapy platform. HiTE™ addresses the unique challenges of NK cell transduction with superior efficiency while preserving NK cytotoxic function and ADCC capacity. Compatible with both primary NK cells and NK-92 cell lines.

iPSC-Derived Cell Therapies

For iPSC-based allogeneic therapies (iPSC-NK, iPSC-T cells), HiTE™ enables 3.6× improved transduction with preserved pluripotency markers.

Cost Component	Traditional	With HiTE™
Viral vector cost/dose	$25,000–$50,000	**$5,000–$10,000**
Vector usage reduction	Baseline	**5–10× reduction**
Total COGS per dose	$95,000–$120,000	**$60,000–$80,000**
Cost savings	—	**33–50%**

Table 5. Representative cost impact analysis*. Actual savings will vary by vector type, MOI, and manufacturing scale.*

Gene Therapy Applications

Beyond immunotherapy, HiTE™ enables efficient transduction for hematopoietic stem cell (HSC) gene therapy, TCR-T cell manufacturing for solid tumor targeting, reporter gene studies and lineage tracing, and CRISPR delivery for multiplex gene editing.

Conclusion

Viral transduction efficiency is not merely a technical performance metric—it is the rate-limiting step that determines the feasibility, cost, and clinical scalability of an entire generation of cell and gene therapies. The inability to reliably transduce primary immune and stem cells at high efficiency and low MOI has imposed structural constraints on manufacturing economics, safety profiles, and patient access that conventional enhancers have been unable to overcome.

HiTE™ changes this equation. As the first Transient Fusion-Promoting Peptide technology, HiTE™ delivers transduction efficiencies of 63% in primary CD3+ T cells, 73.9% in iPSCs, and 33.3% in NK cells—all at low MOI, with cell viability exceeding 90% and VCN maintained well within regulatory safety guidelines.

The implications extend across the cell and gene therapy landscape: reduced viral vector consumption, lower COGS, improved lot release rates, and a safety profile aligned with regulatory expectations. HiTE™ offers a path toward cell therapies that are not only more efficacious and safer, but more affordable—broadening access to transformative treatments for patients who need them most.

Find out more at hitebio.com

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Making Cell Therapies Safer, Efficacious, and Affordable