CATALYZE
Accelerating Translation

Barriers to Advancing In Vivo Gene Therapy

Synthesis of feedback from leading experts

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About This Report

This report summarizes findings on key technical and translational barriers for in vivo gene therapy, with a focus on blood diseases.

The findings in this report emerged from a process convened by the National Heart, Lung, and Blood Institute (NHLBI), which is outlined on the right. We began with expert interviews to define the landscape of current challenges and priorities. Insights from these discussions informed two targeted workshops to generate the actionable recommendations presented in this report. The first workshop focused on therapy development–related barriers, and the second workshop focused on model and assay development–related barriers.

These findings are shared publicly to enable experts in the field of in vivo gene therapy, and those in adjacent fields with relevant expertise, to come together to collaboratively overcome key barriers to advancing in vivo gene therapy.

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1 **Expert Interviews**
22 experts

2 **Workshop 1: Therapy Development**
7 expert participants

3 **Workshop 2: Model & Assay Development**
8 expert participants

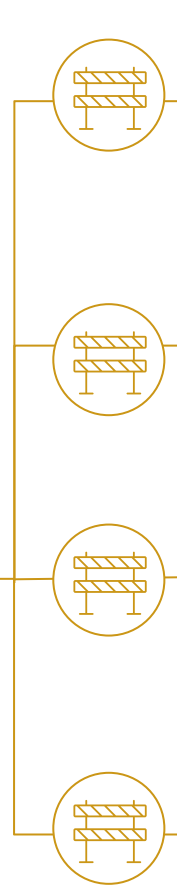
4 **This Report**

Executive Summary

As gene-editing technologies have advanced, in vivo gene therapy progress is bottlenecked by barriers with delivery systems and the ability to predict safety and efficacy of therapies in humans.

Leading in vivo gene therapy experts were engaged in interviews and workshops, and they converged on a set of high-priority barriers to be overcome to advance the field. Feedback indicated:

- The primary constraint is no longer the editing technology; instead, **the dominant bottlenecks are delivery systems and human-relevant safety and efficacy prediction**.
- **There are four key barriers** bottlenecking progress (see next section for additional details on these and other barriers).
- **Cross-disciplinary expertise is needed to solve these barriers.** Expertise from adjacent disciplines should be brought into the in vivo gene therapy community to drive progress.



Unexplained in vitro vs. in vivo mismatch: A fundamental gap exists between how delivery systems behave in cell culture and what happens inside a living organism. Without understanding *why* this mismatch occurs mechanistically, the field cannot rationally bridge the two systems or identify which in vitro findings are worth pursuing in vivo.

Unresolved humanized mouse translatability: Humanized mouse models are the most accessible preclinical system for studying human hematopoietic stem cell (HSC) biology, but they carry critical limitations. Results from these models frequently fail to translate to nonhuman primate (NHP) or clinical settings, and no established framework can predict when or why this divergence occurs.

Foreign protein tolerance unknown: When in vivo gene editing corrects a disease mutation, the patient's immune system may recognize the newly expressed protein as foreign. Current unknowns include whether to immunosuppress, how to assess individual tolerance likelihood pre-dosing, and what thresholds trigger immune clearance. This barrier is distinct from vector immunogenicity and concerns the edited cell product itself.

Niche intercellular crosstalk absent from models: HSCs do not exist in isolation. Their biology is fundamentally shaped by niche cells (i.e., osteoblasts, endothelial cells, megakaryocytes), cytokine gradients, and physical matrix cues. Current in vitro and xenograft models strip away this complexity, making them poor predictors of in vivo editing outcomes, engraftment efficiency, and cell fate after editing.

Key Barriers

to advancing in vivo gene therapy

Experts converged on a short list of high priority barriers.

The following selection counts are from individual expert work periods during the two workshops.

Focus of synthesis: Due to the high convergence, needs associated with these four barriers are the focus of synthesis slides.

These barriers received **fewer selections**. However, they are interconnected with the top four barriers and represent enabling knowledge gaps.

Barriers	Selected as a top priority by…		Workshop
Unexplained in vitro vs. in vivo mismatch	**75%**	6 of 8 experts	WS2
Unresolved humanized mouse translatability	**63%**	5 of 8 experts	WS2
Foreign protein tolerance unknown	**57%**	4 of 7 experts	WS1
Niche intercellular crosstalk absent from models	**50%**	4 of 8 experts	WS2
HSC uptake mechanism unknown	**43%**	3 of 7 experts	WS1
Disease-state / cell cycle chromatin landscape unmapped			WS1
Immunotoxicity response to delivery vehicles unpredictable			WS1
HSC transfactor content unmapped	**38%**	3 of 8 experts	WS2
No SCD large-animal model			WS2

Barrier Category Key: Bioengineering | In Vivo Biology | Gene Architecture

Top Selected Barriers:

1. **Unexplained in vitro vs. in vivo mismatch**
2. Unresolved humanized mouse translatability
3. Foreign protein tolerance unknown
4. Niche intercellular crosstalk absent from models
5-9. Other highly prioritized barriers

Unexplained In Vitro vs. In Vivo Mismatch

Core Problem: A fundamental gap exists between how delivery systems behave in cell culture and what happens inside a living organism. Without understanding *why* this mismatch occurs mechanistically, the field cannot rationally bridge the two systems or identify which in vitro findings are worth pursuing in vivo.

Expert Consensus & Key Trends:

- Experts broadly agree that in vitro success is necessary but not sufficient. Moreover, in vitro models can be actively misleading when off-target sequestration by liver, lung, and spleen is not modeled.
- Flow conditions (e.g., particle disassembly dynamics, 3D tissue architecture) are all absent from standard in vitro systems and cannot be replicated simply by adding complexity to a dish.
- HSC activation state and cell cycle status are critical variables: cells that cycle readily in vitro may not cycle in vivo, directly limiting HDR-based editing strategies.
- The field lacks a productive middle-ground model between in vitro screening and expensive NHP studies; diverse animal models (e.g., mouse, pig, NHP) tested in parallel represent a near-term path to building a translational "Rosetta stone."
- Data standardization across labs is a prerequisite for cross-study comparison; different protocols and sequencing methods make existing data sets difficult to reconcile.

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Unexplained In Vitro vs. In Vivo Mismatch

Expert Quotes:

> "I kind of feel like success in vitro is sort of necessary, but not sufficient and sometimes very misleading... targeting isn't [similar enough to in vivo]—it is not just on target targeting, it's also an ability to ignore all the very seductive off targets... a lot of [in vitro models] are just nonspecific, such as physical sequestration and organs like the lungs and the liver that like to grab fatty molecules or viral particles."
>
> Workshop 2 Participant

> "In the lipid nanoparticle world, its opposite—things [that] don't work in vitro, sometimes they work really well in vivo. And they might work in vitro and don't do anything in vivo ... there are certain serum proteins that bind, especially with lipid nanoparticles like APOE, which might be absent in the media."
>
> Workshop 2 Participant

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Unexplained In Vitro vs. In Vivo Mismatch

Actionable opportunities to overcome this barrier:

i. Invest in mechanistic biodistribution studies across diverse animal models to build a cross-species translation database.

ii. Pair biodistribution studies with organoid models to identify which in vitro findings have predictive value before moving to larger animals.

iii. Develop shared assay standards and reporting protocols so that in vitro and in vivo data from different labs can be compared and aggregated.

iv. Prioritize HSC-specific models that account for disease state (SCD inflammatory milieu) and cell cycle status—two variables that existing in vitro systems routinely miss.

v. Explore pigs and other large nonrodent animals as intermediate-throughput models that fill the gap between mouse and NHP.

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Unresolved Humanized Mouse Translatability

Core Problem:

Humanized mouse models are the most accessible preclinical system for studying human HSC biology, but they carry critical limitations. Results from these models frequently fail to translate to NHP or clinical settings, and no established framework can predict when or why this divergence occurs.

Expert Consensus and Key Trends:

- In Workshop 2, this barrier produced the single highest consensus, reflecting broad expert agreement that humanized mice cannot adequately model the human immune response to in vivo delivery systems.
- NHP models are widely regarded as superior, but they are largely cost-prohibitive, making broad field access untenable.
- A commonly used protocol (humanizing mice, waiting ~4 months for full engraftment, then injecting delivery vehicles) likely fails because HSC surface markers such as C-kit are downregulated within 2 weeks of engraftment, meaning the targeting ligand is effectively absent at the time of injection.
- Transgenic humanized mice expressing human cytokines (e.g., IL-15 for improved NK cell reconstitution) represent a near-term improvement, but access to and distribution of these models is a bottleneck in itself.
- No model will ever be perfect; the field must develop decision frameworks for when existing models are sufficient to justify clinical advancement.

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Unresolved Humanized Mouse Translatability

Expert Quotes:

> "A simple change [shortening the time of humanization in the mouse model] could help make the humanized model a little bit more helpful, even though I still think it's not optimal. Obviously, there's no immune system. The macrophage system is there, but not fully functional either. So, what we see in the monkey would not necessarily be detectable in a humanized mouse. So, I think there is clearly work to be done."
>
> Workshop 2 Participant

> "Post-COVID, they've gone up about 2.5x in price, just meaning that's a tough model for the field to be able to get access to and to utilize as a whole. So, you know, even so, there's some lack of translatability, especially in immune responses in monkey versus human. You know, the cytokine storm was not well predicted in nonhuman primate … But if we could develop enough data, or Rosetta stone, to be able to say, oh, if we see this in the mouse, it means we see it in the human."
>
> Workshop 2 Participant

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Unresolved Humanized Mouse Translatability

Actionable opportunities to overcome this barrier *(not exhaustive)*:

i. Establish centralized sources or distribution agreements for transgenic cytokine-humanized mouse models; decentralized, lab-by-lab access is a structural bottleneck to field-wide adoption.

ii. Shorten humanization time windows: engrafting cells and testing delivery vectors within 1–2 weeks—when HSC surface markers are still expressed—could make current humanized mouse protocols meaningfully more informative.

iii. Develop explicit model-to-model translation criteria: what findings in humanized mouse warrant advancement to NHP, and what NHP findings are sufficient to justify IND filing?

iv. Explore high-throughput in vivo screening approaches (e.g., barcoded particle libraries tested in small animals) to reduce reliance on NHP for early-stage candidate selection.

v. Treat negative NHP findings as field-level data—create incentive structures or a shared repository for NHP results that are currently unpublished, including from industry.

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Foreign Protein Tolerance Unknown

Core Problem:

When in vivo gene editing corrects a disease mutation, the patient's immune system may recognize the newly expressed protein as foreign. Current unknowns include whether to immunosuppress, how to assess individual tolerance likelihood predosing, and what thresholds trigger immune clearance. This barrier is distinct from vector immunogenicity and concerns the edited cell product itself.

Expert Consensus and Key Trends:

- The experts agree that this is a distinct and underappreciated barrier that intersects immunology and clinical translation.
- No validated animal model currently exists for predicting human immune tolerance to gene-edited cell products.
- HSCs as antigen-presenting cells remain poorly characterized; whether an HSC expressing a newly corrected protein can present that antigen to the adaptive immune system is a fundamental unknown.
- CRIM status (whether a patient has ever expressed any version of the target protein) is a known modifier of immune tolerance risk, but no predosing assay exists to stratify patients by tolerization likelihood.
- The problem is distinct from payload silencing: here, the editing succeeds and the protein is expressed, but it is then recognized as foreign and cleared.

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Foreign Protein Tolerance Unknown

Expert Quotes:

> "If you deliver something violently immunogenic, it will have consequences. But what happens if you deliver something to the LT-HSC and the target, as Mike Holmes said, from other tissues? Are we worried? How worried are we about the immune response?"
>
> Workshop 1 Participant

> "Immunogenicity I guess could be at a lot of different levels—it could be immune responses that prevent it from getting to the cell in the first place, or maybe it does get to the cell, but the patient has a systemic immune response that's detrimental, you know, regardless—or maybe if its just a short lived pulse, you know, it may just be an immune response that lasts for the duration of the material being present. But if there's permanent genetic change, you know, could there be an enduring immune response?"
>
> Workshop 1 Participant

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Foreign Protein Tolerance Unknown

Actionable opportunities to overcome this barrier *(not exhaustive)*:

i. Develop a predosing tolerization assessment framework that accounts for cross-reactive immunological material (CRIM) status. This framework should identify which patients are likely to mount an immune response against the newly expressed protein before dosing.

ii. Clarify the neoantigen risk in HSC-specific contexts. HSCs are known to present antigens via major histocompatibility complex (MHC) class I and II, but whether corrected HSCs will trigger immune clearance of the edited cell pool in a clinical conditioning context is unresolved. This is a distinct question from vector immunogenicity and warrants dedicated mechanistic study.

iii. Develop models that capture patient-specific immune tolerance variation. This barrier may require models that reflect individual patient differences.

iv. Incorporate foreign protein tolerance as an explicit endpoint in programs (e.g., Catalyze preclinical support packages) that involve correction of loss-of-function mutations in CRIM-negative disease contexts.

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Niche Intercellular Crosstalk Absent from Models

Core Problem:

HSCs do not exist in isolation. Their biology is fundamentally shaped by niche cells (i.e., osteoblasts, endothelial cells, megakaryocytes), cytokine gradients, and physical matrix cues. Current in vitro and xenograft models strip away this complexity, making them poor predictors of in vivo editing outcomes, engraftment efficiency, and cell fate after editing.

Expert Consensus and Key Trends:

- Experts strongly agree that 2D and 3D organoid models fundamentally lack the crosstalk needed to predict HSC permissiveness and engraftment—and that even xenograft models involve a mismatch between human HSCs and a murine niche.
- Clinical trials using HDR editing approaches have shown unexpectedly poor and delayed engraftment, raising the possibility that the editing process itself disrupts niche-dependent cell fate decisions in ways not captured by standard models.
- Disease-state distortion of the niche—the SCD inflammatory milieu, for example—is a second layer of complexity that is almost entirely absent from current models; preconditioning (e.g., transfusion status before HSC collection) may modify the niche in ways that affect in vivo editing outcomes.
- Microfluidics and semiconductor-derived organ-chip platforms represent an adjacent technology that could enable physiologically relevant niche modeling but have not been systematically applied to HSC biology.
- Experts debated the best near-term path: cytokine-transgenic humanized mice that support human niche cells vs. microfluidic bone marrow chip platforms.

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Niche Intercellular Crosstalk Absent from Models

Expert Quotes:

> "Is there a way to bring people together from different industries to sort of resolve [this crosstalk challenge] if there is no way we can come up with like an in vivo model... you know, the chip industry can put a lot of these things together and [have] multiple flows going on."
>
> Workshop 2 Participant

> "One of the things that I've been thinking a lot about in this kind of HSC therapeutic genome editing field is about how editing affects stem cell function and then engraftment of these hematopoietic stem cells. … There's been clinical trials of for example, HDR in HSCs, where after editing with a nuclease and AAV vector resulted in basically poor and delayed engraftment in patients, and so are there more physiologic models that might be able to predict this?"
>
> Workshop 2 Participant

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Niche Intercellular Crosstalk Absent from Models

Actionable opportunities to overcome this barrier *(not exhaustive)*:

- Characterize the specific niche signals missing from humanized mouse models before designing new model systems; the problem cannot be fixed until it has been well defined.
- Engage microfluidics and semiconductor engineers as adjacent field experts who can contribute organ-chip design capabilities that the gene therapy field lacks.
- Incorporate disease-state niche biology as a required modeling variable: SCD inflammatory milieu that structurally alter the niche must be represented in models used to evaluate in vivo editing approaches.
- Treat the SCD preconditioning question (transfusion status, timing before HSC engagement) as a near-term mechanistic study opportunity—the variable exists in current clinical workflows, and the data required to study it may already be accessible.

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5-9 **Other highly prioritized barriers**

Five additional barriers were identified as important but with a lower consensus (I / III).

Unknown HSC uptake mechanism

- *(Directly enables Barrier #1)* Resolving uptake biology is a prerequisite to interpreting why in vitro success fails to predict in vivo delivery.
- Experts noted that high-throughput screening of internalizing receptors and endocytic pathways in HSCs is a tractable near-term goal.
- However, single-cell resolution measurements of uptake mechanisms have not been systematically applied.
- Distinguishing endocytosis pathways in off-target tissues from those in HSCs enables improved identification of targeting ligands and delivery vehicle design.

Unmapped disease-state / cell cycle chromatin landscape

- *(Enables Barriers #1 and #3)* Understanding chromatin accessibility enables better models and improved post-edit protein expression prediction.
- Chromatin accessibility in HSCs is dynamic, yet editing programs are designed against chromatin reference maps generated that do not reflect these dynamics.
- Experts highlighted that the epigenomic environment is altered by SCD and other disease states.
- Cell cycle quiescence in vivo versus activation ex vivo represents a particularly consequential and largely uncharacterized variable.

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Five additional barriers were identified as important but with a lower consensus (II / III).

Unpredictable immunotoxicity response to delivery vehicles

- *(Enables Barriers #2 and #3)* Immune response prediction is a shared prerequisite for humanized mouse model validity and foreign protein tolerance assessment.
- Immune response to delivery vehicles cannot be reliably predicted from any single preclinical model.
- Experts identified a fundamental 3-fold problem:
  1. Humanized mouse models are insufficient to model delivery efficiency.
  2. NHP models are closer but cost-prohibitive.
  3. In vitro model systems are comparatively inexpensive but lack immune complexity.

Unmapped HSC transfactor content

- *(Foundational to all nine barriers)* Resolving cellular regulatory pathways affects a broad set of barriers.
- The transcription factor landscape controlling HSCs has not been systematically characterized across disease contexts or patient populations.
- Experts identified this as one of the most foundational knowledge gaps in the field.
- Without a comprehensive atlas of the HSC transcriptome and proteome, predictions about gene therapy performance are limited.

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Five additional barriers were identified as important but with a lower consensus (III / III).

No SCD large-animal model

- *(Enables Barriers #1, #2, and #4)* Resolving delivery mismatch, mouse model translatability, and niche crosstalk all require a disease-state large-animal model.
- No NHP model carries the sickle cell mutation, meaning NHP studies of in vivo HSC editing reflect healthy-animal biology rather than SCD pathophysiology.
- The field therefore lacks a broadly accessible, disease-state large-animal model.

Other key takeaways from expert consensus (cross-cutting):

- Model inadequacy is the dominant field-wide constraint.
- Disease-state biology is systematically underrepresented.
- Several high-priority barriers require disciplines that are not currently at the table (e.g., microfluidic engineering, chromatin biology, genotoxicity risk modeling).