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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

Cellular immunotherapies: targeting new frontiers

GUEST EDITOR

Yu Cao

CELL & GENE THERAPY INSIGHTS

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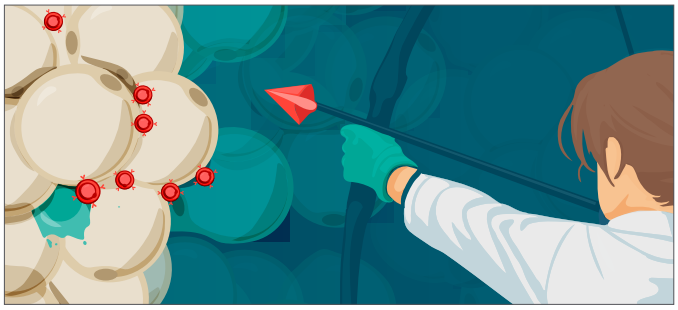

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CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

The next era of cell therapy

Yu Cao

EDITORIAL

"...the next era of cell therapy will be defined less by isolated breakthroughs and more by integration."

Advanced cell therapy has entered a period of explosive growth. The past decade proved that engineered immune cells can cure diseases once considered untreatable. The next decade will determine whether those breakthroughs can overcome current limitations and mature into foundational pillars of medicine. We are now at a moment when biological possibility is no longer the primary constraint. Instead, the field must solve the intertwined challenges of manufacturing, delivery, safety, systems integration, and global scalability.

Across the contributions in this edition, a unifying theme emerges: advanced cell therapy is evolving from a collection of extraordinary scientific achievements into an industrial, regulatory, and clinical ecosystem that must function as a coordinated whole.

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FROM *EX VIVO* SUCCESS TO *IN VIVO* AMBITION

Ex vivo CAR-T therapies have achieved durable remission, and in some cases cure, in hematologic malignancies. Yet their success also exposed the accessibility limits of individualized manufacturing. High cost, long production timelines, and logistical complexity restrict access and prevent scale.

In vivo CAR-T approaches aim to convert the body itself into the manufacturing site. Rather than shipping cells across

continents, viral vectors or nanoparticles deliver genetic instructions or mRNA directly to immune cells inside the patient. This paradigm promises off-the-shelf availability, outpatient delivery, and a fundamentally different economic model. But promise alone does not guarantee translation.

The **commentary by Adrian Bot and Xianghong Li**, along with my conversation with **Shon Green**, captures both the excitement and the caution surrounding this modality [1,2]. Early clinical signals show that *in vivo* CAR-T generation can achieve meaningful biological effects, yet the field must confront unresolved questions: genomic integration risk, dosing control, redosing feasibility, immunogenicity, and long-term persistence. What becomes clear is that *in vivo* CAR-T is not simply a new tool – it represents a new category of medicine that demands new frameworks for modeling, analytics, and clinical evaluation.

MANUFACTURING AS THE NEW BOTTLENECK

If the first wave of *in vivo* cell therapy was constrained by biology, the second wave is constrained by manufacturing maturity. **Dehui Kong**'s analysis of CMC considerations makes a critical point: scalability is not an afterthought; it is the rate-limiting step. Both viral vectors and non-viral mRNA-LNP systems face persistent challenges related to production complexity, cost, and regulatory burden [3]. She argues that CMC maturity, rather than biological feasibility, may be the decisive factor shaping *in vivo* CAR-T platforms.

For *ex vivo* allogeneic cell therapies, cryopreservation and post-thaw functional stability represent an additional, often underappreciated manufacturing constraint, as described by **Jason Acker** [4]. Without reliable preservation strategies that maintain potency across storage, transport, and clinical deployment, even scalable production risks collapsing at the final translational step.

Reagan Jarvis reframes these challenges as one of systemization. The future success of cell therapies depends less on incremental scientific breakthroughs and more on disciplined integration of discovery and manufacturing [5]. **Shon Green's interview** echoes this reality from a developer's perspective: analytics, potency assays, and comparability frameworks remain immature for *in vivo* CAR-T products. Without robust measurement systems, even the most elegant platforms risk regulatory and translational stagnation [2].

SAFETY & SUPPORTIVE INFRASTRUCTURE

As cellular therapies grow more powerful, supportive care becomes a central enabling technology. **Liam Tremble and Paula Maguire's review** reminds us that CRS, ICANS, and other therapy-induced toxicities are not peripheral complications; they are predictable consequences of immune activation [6]. Supportive care innovation is therefore not separate from therapeutic progress; it is a prerequisite for it. Regulatory frameworks, incentives, and dedicated drug development pathways must evolve in parallel with cell therapy innovation.

SOLID TUMORS & BEYOND ONCOLOGY

Arnaud Deladeriere argues that the persistent challenges of CAR-T and related modalities in solid tumors reflect not only biological barriers but also translational and systemic shortcomings [7]. Durable success in solid tumors will require integrated strategies that align biological design with manufacturability, intellectual property frameworks, and commercial realism.

While oncology remains the proving ground, the most transformative

implications of CAR-T may lie beyond cancer. Autoimmune disease, fibrosis, metabolic disorders, and allergy represent emerging frontiers where immune reset strategies could redefine chronic disease management. Several authors emphasize that transient immune reprogramming may be sufficient to induce durable remission without lifelong treatment. This reframes cell therapy from a last-resort oncology intervention into a platform technology for systemic disease modification.

CHINA BIOTECH ECOSYSTEMS & CONVERGENCE

Two interviews in this issue explore how geopolitical and ecosystem differences shape innovation trajectories. In my conversation with **Zhenghong Gao**, he highlights China's biotech landscape as a complementary model optimized for rapid clinical translation and manufacturing scale [8]. Investigator-initiated trial frameworks, regulatory agility, and coordinated infrastructure enable faster iteration and proof-of-concept generation.

Rather than framing East and West as competitors, both Zhenghong and Shon point toward a future defined by convergence. The USA and EU excel at high-risk pioneering science; China excels at rapid execution and scale. The global future of advanced therapies may depend on how effectively these strengths interact. In a field where development timelines span a decade and patient need is immediate, speed is not a luxury but an ethical obligation.

EPILOGUE

If this special issue carries a single message, it is this: the next era of cell therapy will be defined less by isolated breakthroughs and more by integration. Biology, manufacturing, analytics, regulatory strategy, and global collaboration must advance together. Fragmented innovation may not deliver scalable medicine.

The field is transitioning from a phase of heroic experimentation to one of disciplined construction. That transition is difficult but necessary. Success will depend on assembling scientific and industrial components into a coherent system capable of reaching patients everywhere, not only in specialized centers.

The science has already proven what is possible. The next challenge is proving what is practical. And that challenge is no less ambitious.

REFERENCES

1. Bot A, Li X. *In vivo* chimeric antigen receptor (CAR) therapy: quo vadis? *Cell Gene Ther. Insights* 2026; 12(1), 87–92.
2. Green S. The next frontier of *in vivo* CAR-T: a conversation with Shon Green. *Cell Gene Ther. Insights* 2026; 12(1), 15–22.
3. Kong D. CMC considerations on *in vivo* CAR-T. *Cell Gene Ther. Insights* 2026; 12(1), 1–5.
4. Acker J. The cryo-sensitivity of NK cells: overcoming post-thaw decline. *Cell Gene Ther. Insights* 2026; 12(1), 97–104.
5. Jarvis R. Systemization is key to fulfilling T cell therapy's promise in cancer. *Cell Gene Ther. Insights* 2026; 12(1), 105–109.
6. Tremble L, Maguire P. Role of supportive care in the future of cell therapies. *Cell Gene Ther. Insights* 2026; 12(1), 81–86.
7. Deladeriere A. Solid tumors and advanced therapies: why science alone will not deliver the cure. *Cell Gene Ther. Insights* 2026; 12(1), 155–162
8. Gao Z. Leaping forward: how China's biotech evolution is capturing the *in vivo* CAR-T frontier. *Cell Gene Ther. Insights* 2026; 12(1), 7–12.

BIOGRAPHY

Yu (Clay) Cao PhD is an accomplished scientific leader with over 15 years of experience in drug discovery and translational development. Currently serving as Vice President and Head of Drug Discovery at GenEditBio, Dr Cao leads cross-functional teams advancing cell and gene therapies across rare diseases, oncology, and neurodegenerative disorders. He has deep expertise in preclinical development, IND-enabling studies, and *in vivo* delivery technologies, with a strong track record of progressing novel cell and gene therapeutics from discovery to clinical stage. Prior roles include research leader roles at Tessera Therapeutics and Agios Pharmaceuticals, where he drove innovative programs in immuno-oncology and gene editing. Trained as an immunologist, Dr Cao holds a PhD in Immunology, with additional credentials from Harvard Business School and Johns Hopkins University. He actively contributes to the field as an advisor or leadership member of different non-profit organizations.

Yu (Clay) Cao, Vice President and Head of Drug Discovery, GenEditBio, Boston, MA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNOTHERAPIES:
TARGETING NEW FRONTIERS

SPOTLIGHT

COMMENTARY

In vivo CAR therapy: quo vadis?

Adrian Bot and Xianghong Li

In vivo CAR therapies are rapidly emerging as a strategy to address the scalability, access, and toxicity limitations associated with conventional *ex vivo* CAR-T cell products. This article reviews viral and LNP–RNA platform technologies, emerging clinical evidence, and key safety and regulatory considerations relative to established CAR-T and immune cell engager approaches. While early progress is encouraging, careful translational evaluation is essential to determine whether *in vivo* CAR strategies can ultimately meet or surpass current standards of care across oncology, autoimmunity, and other indications.

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Last decade witnessed the advent of engineered T cell therapies to the therapeutic armamentarium in oncology, through several approved autologous CAR and TCR-engineered cell products [1] and some exciting emerging evidence of CD19 and BCMA-directed CAR-T cell efficacy in B cell involved autoimmune disorders [2]. Amongst the lessons learned to date, especially with viral-engineered CAR-T cell products, the remarkable efficacy in certain B cell malignancies translating to cures in many patients stands out. Nevertheless, scalability, patient access limitations, and toxicities owing to utilization of lymphodepletion conditioning, difficult-to-predict or manage on target toxicities, and caveats related to utilization of genomically integrated viral vectors, ignited considerable interest in novel technologies. Notably, less than 30% of eligible patients with large B cell lymphoma (LBCL) receive CAR-T treatment, largely due to manufacturing constraints and rapid disease progression during the waiting period.

Hence, major efforts have been deployed during the last few years in bringing together the unprecedented potency afforded by CAR-T therapy with the scalability of engineered viral vectors and RNA-nanomedicines for direct *in vivo* engineering of immune cells [3]. *In vivo* CARs are being developed to overcome these limitations by shifting the manufacturing burden away from complex, patient-specific cell processing towards standard drug manufacturing, aiming for both off-the-shelf availability and applicability to outpatient setting thereby augmenting access. These translated into a rapidly expanding ecosystem of likely more than 35 biotechnology companies operating predominantly

in China and the US, with at least eight of them reaching a clinical stage. The two main technologies are based on engineered lentiviral vectors modified for selective cell targeting, and RNA formulated in lipid nanoparticles (LNPs) targeted mostly through antibody-based functionalization to enable select lymphocytes engineering – with key features, advantages and limitations described in detail elsewhere [3]. This remarkable progress catalyzed by the involvement of large pharmaceutical companies through strategic partnerships or merger acquisitions announced predominantly during the last two years will likely result in an even more rapid pace of expansion of this field.

Based on the work publicized to date, several themes are emerging. First, by utilizing optimized viral or LNP–RNA platforms, one can achieve a biologically and potentially clinically relevant level of CAR engineering of immune cells *in vivo* [4,5]. While this is limited for now to B cell lineage/plasma cell antigens in multiple myeloma and lupus respectively, the rapid kinetics of CAR-engineering and of the pharmacologic effect are notable. There is also emerging clinical proof of biology with a macrophage-tropic nanoformulation loaded with an anti-Trop2 CAR in mRNA format in solid tumors. Secondly, from a safety perspective, a profound pharmacologic effect is accompanied by toxicities – similar to conventional *ex vivo* engineered CAR-T cell products – that can be managed utilizing low dose corticosteroids and IL-6R blockade. In fact, pre-emptive utilization of low dose dexamethasone is quite prevalent for LNP–RNA platforms to preempt exaggerated acute phase response owing to infusion of nanoparticles. Third, it is feasible and safe to repeat infusions of LNP–RNA formulations over a limited interval, to generate sufficiently large and sustained CAR-T cell populations for a pharmacologic effect to occur. Fourth, utilization of non-human primates to guide the evaluation, optimization of individual platforms and even product candidates – both engineered lentiviral vectors and LNP–RNAs – has been critically enabling for successful clinical translation. Fifth, there are diverse development and regulatory options for industry sponsors based on ecosystems with different clinical entry bars (China: lower, Australia: intermediate, and North America: higher) and availability of global contract research and manufacturing organizations supporting nimble insourcing/outsourcing strategies. Finally, the rather boisterous advent of *in vivo* CAR-Technologies and the continuous progress with immune cell engagers, is shifting the attention of investors and large biopharma away from conventional *ex vivo* engineered immune cell products carrying a negative economic impact with respect to the latter.

But is this veritable 'gold rush' solidly supported by the evidence to date, or do we need more clinical experience with *in vivo* CARs in B cell disorders and across broader disease populations? One particular concern with *in vivo* genomically integrating CAR payloads relates to uncontrollable persistence or secondary expansion of functional CAR-expressing immune cells leading to protracted or recurring on target toxicities that may require up to 15 years patient monitoring **(Table 1)**. More specifically, for lentiviral vectors, the concern of genotoxicity stems from the potential risk of insertional mutagenesis, as the CAR gene randomly inserts into the host cell genome at site that could activate oncogenes or disrupt tumor suppressor genes, events that may trigger or facilitate a secondary malignant process. Safety monitoring should include clonal tracking, integration-site analysis, and evaluation of unexpected cell phenotypes. Emerging genomic integrating technologies that afford targeted or preferential integration sites with insulating elements and/or that carry controllable CARs (with suicide/depletion tags; small-molecule based

inducible ON/OFF switches) may overcome some of these challenges. In turn, while dose-tunable and devoid of genotoxicity, LNP-based formulations with transiently expressed constructs off RNA payloads present a range of other challenges from engineering efficiency to liver-tropism and immunogenicity – that may preclude redosing as discussed below.

Depending on these factors, we could envision a lower entry bar for the genomic integrating vector technologies in oncology as compared to autoimmunity. Conversely, the tunability and transiency afforded by RNA based *in vivo* CAR platforms may be an advantage in indications associated with high safety bar (autoimmunity). Nevertheless, key questions need to be answered with the LNP–RNA platform, including possibility to yield desired levels of CAR cell engineering more reproducibly for a durable pharmacologic effect to occur. Utilization and optimization of the targeted LNP–RNA platform, and re-dosing without immunogenicity risks seem to be critically important; time will tell whether the technologies currently in early clinical development stage meet this bar. Over-arching questions related to the broader competitive landscape and include whether the current *in vivo* CAR technologies match the potency of currently available *ex vivo* viral-engineered CAR-T cell products benefiting from a highly optimized treatment regimen including pre-conditioning by lymphodepletion. To fully displace conventional CAR-T cell products, *in vivo* approaches must meet comparable or superior clinical performance bars. Hence, we anticipate that the first wave of *in vivo* products will likely be positioned in disease indications associated with a higher safety bar but lower threshold for efficacy. Methods to augment immune cell fitness *in vivo* may greatly enable this treatment modality in particular indications where this is a limiting factor. Another question is whether *in vivo* CAR therapies in any format may carry potency, dose tunability, or safety advantages over the rapidly evolving arena of recombinant immune cell engagers. In oncology indications, in earlier lines, definition of patients at high risk for relapsing post standard of care including conventional CAR-T cell intervention – for example through monitoring minimal residual disease (MRD) – would provide an opportunity to accelerate novel treatment modalities (e.g., *in vivo* CAR-Therapies, immune cell engagers) for consolidative/curative purpose especially, particularly if their safety profile is more favorable owing to lack of chemo-based lymphodepletion These questions are likely to influence the interest and resourcing behind life cycle management of current conventional CAR-T cell technologies versus *in vivo* CAR platforms and immune cell engagers, respectively. Despite scalability hurdles, a rapid sunset for *ex vivo* engineered CAR-T cell products may not occur unless these novel technologies match their clinical efficacy.

Based on the considerations discussed above and the novelty of this area, a comprehensive translational approach will be key to characterize in detail the strengths and shortcomings of *in vivo* CAR technologies currently deployed in clinic or nearing clinical stage. Together with other aspects such as manufacturing scalability and cost of goods, this will help guide product development towards registration or back to bench for additional optimizations. For integrating viral based vectors, it will be important to continue to drive towards achieving exquisite tissue and cell selectivity, avoiding off-target tissue or cell uptake and engineering. Genotoxicity could severely limit broad utilization of this emerging modality. For LNP–RNA approaches, minimizing liver tropism, undesired uptake by macrophages, and immunogenicity will be important to enable chronic therapies safely in an outpatient setting.

Particular attention needs to be dedicated to product immunogenicity

monitoring and mitigation planning. Innate responses, liver tropism, complement activation, and dsRNA-sensing of residual dsRNA or other immunogenic impurities may drive acute infusion reactions and transient laboratory changes (e.g., changes in liver enzymes). In addition, adaptive immune responses may include anti-viral vector or LNP formulation antibodies and/or anti-CAR construct immunity that may limit the treatment efficacy or re-dosing. Finally, pre-existing anti-PEG antibodies may pose challenges for select technologies.

Next-generation approaches for *in vivo* CAR engineering in particular, and immune system reprogramming in general, will need to integrate the strengths of currently explored modalities and dial out the liabilities (Table 1). Exquisitely precise payload delivery, gene modifications including CAR sequence insertion, and RNA-based gene writing, coupled with novel features allowing tunability and spatiotemporal control of expression, will likely catalyze an expansion of molecular and cellular targets, payload architectures, and clinical indications pursued. In oncology, there are two interesting paths: first, in indications with low burden or MRD, could a safe and tunable *in vivo* CAR approach achieve the pharmacological effect to eradicate all the residual cancer cells likely disseminated throughout the body? Second, in case of bulky tumoral processes, can one orchestrate through multifaceted *in vivo* immune system engineering, an attack that would drive both tumor resolution and pre-empt clonal evasion mechanisms? Would there be a need to co-target the stroma, reprogram the tumor microenvironment, block, or exploit in other ways the numerous immune checkpoints, or co-opt broader mechanisms to achieve a meaningfully deep and durable response? Can these be achieved by *in vivo* immune engineering utilizing a larger spectrum of tools?

In indications outside oncology such as autoimmunity, major opportunities will be afforded by exploiting the mechanism of 'immune reset' aimed to impart durable, drug-free responses [2]. This generally involves the transient yet global (blood and tissues) elimination of all immune

TABLE 1

Paving the way to ***in vivo*** engineering of the immune system.

Current technologies		Next-generation technologies
• Unknown clinical safety profile owing to integration of different technologies never explored together in clinic • Uncertain competitive advantages over immune cell engagers or optimized cell therapies		• Vectors or formulations with exquisite tissue and cell selectivity, avoiding off target tissue / target cell uptake; this includes next-generation targeted LNPs and lentiviral vectors, improved for tissue and cell tropism • Diversified categories of payloads (multiplexed CAR/TCRs, biological response modifiers, checkpoints, gene editing, gene writing systems) • Payload designs allowing prolonged RNA payload expression, precise genomic integration and/or enhanced spatial-temporal control of expression • Novel technologies with enhanced tunability achieving desired exposure over time without cellular, genomic sequelae or immune reactions • Advances with analytical characterization, preclinical, clinical pharmacology analysis and regulatory environment, leading to streamlined development pathways
Lentiviral vectors • Relatively stochastic integration sites • Potentially difficult monitoring for chronic or recurring toxicities • Possible off-target cell transduction in cells such as macrophages or target cells • Uncertain applicability to indications with ↑ safety bar (e.g. autoimmunity) • Manufacturing hurdles (scale up, product characterization)	**RNA–LNP format** • Transfection limited by uptake, endo-lysosomal escape, translational capacity, or other mechanisms • Transient engineering profile; unknown high potency, fitting indications with ↑ efficacy bar • Possible off-target cell uptake; liver and myeloid cells tropism • Potential immunogenicity; uncertainty whether it can be administered chronically	

LNP: lipid nanoparticle; TCR: T cell receptor; CAR: chimeric antigen receptor.

cells including autoreactive memory cells, followed by the regeneration of a normally functioning immune system largely devoid of pathogenic immune cells. This can result, at least in select disease indications, in a prolonged clinical benefit and even partial or complete recovery or organs functional capacity without requiring concomitant treatments. Nevertheless, as the field is nascent, major questions still need to be answered, including whether a transient yet global B cell depletion, for example, is sufficient to impart a drug-free durable response, especially in disease indications where predisposing genetic factors or other immune populations co-drive the pathogenesis. Hence, will it be possible to 'reset' other components of the immune system such as T cells and even innate immunity, and/or co-deploy counter-regulatory mechanisms such as T regulatory cells with applicability beyond autoimmune disorders and transplantation? Finally, will it be possible to correct, *in vivo*, genetic defects with strong phenotypic penetrance, which predispose subjects to serious immunological disorders? The therapeutic tools primarily exploited in oncology and autoimmune settings, could ultimately be applicable across a much broader realm of indications including regenerative medicine, heralding the age of this new treatment modality: *in vivo* engineering of cells and tissues.

REFERENCES

1. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR-T cell immunotherapy for human cancer. *Science* 2018; 1361–1365.
2. Muller F, Taubmann J, Bucci L, *et al*. CD19 CAR-T cell therapy in autoimmune disease – a case series with follow-up. *N. Engl. J. Med.* 2024; 390, 687–700.
3. Bot A, Scharenberg A, Friedman K, *et al*. *In vivo* chimeric antigen receptor (CAR)-T cell therapy. *Nat. Rev. Drug Discov*. 2025.
4. Xu J, Liu L, Parone P, *et al*. *In-vivo* B cell maturation antigen CAR-T cell therapy for relapsed or refractory multiple myeloma. *Lancet* 2025; 406, 228–231.
5. Wang Q, Xiao ZX, Zheng X, *et al*. *In vivo* CD19 CAR-T cell therapy for refractory systemic lupus erythematosus. *N. Engl. J. Med.* 2025; 393, 1542–1544.

AFFILIATIONS

Adrian Bot PhD, Executive advisor, NewCo, Beverly Hills, CA, USA

Xianghong Li PhD, Consultant, San Diego, CA, USA

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

EXPERT INSIGHT

The cryo-sensitivity of NK cells: overcoming post-thaw decline

Jason P Acker

The clinical translation of natural killer (NK) cell-based immunotherapies is fundamentally constrained by the lack of optimized cryopreservation protocols. While many hematopoietic cells tolerate 'standard' freezing methods, NK cells exhibit unique sensitivities that result in highly variable post-thaw recovery and diminished cytotoxicity. This review analyzes the biophysical principles of cryobiology, the molecular vulnerabilities of NK cells, and the critical limitations of traditional slow-cooling and DMSO-based techniques. Leveraging advances in new cryoprotectant technologies and targeted molecular tools, specialized, NK-specific cryopreservation strategies are emerging. Advances in automated workflows for NK cell production and pre-/post-cryopreservation handling are addressing critical roadblocks in NK cell manufacturing. Collectively, these advancements in NK cell cryopreservation are paving the way for scalable, 'off-the-shelf' allogeneic immunotherapies that can be manufactured centrally and delivered immediately to patients, bypassing the logistical delays of traditional cell-based treatments.

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The integration of natural killer (NK) cells into the modern oncology toolkit represents an exciting evolution in adoptive immunotherapy. Unlike T cell-based therapies, which often require complex genetic modifications to achieve specificity and are constrained by the risk of graft-versus-host disease (GVHD) in allogeneic settings, NK cells offer a unique innate ability to eliminate malignant cells through a sophisticated balance of inhibitory and activating signals [1]. This intrinsic cytolytic capacity, independent of major histocompatibility complex (MHC) restriction, positions NK cells as the ideal candidate for 'off-the-shelf' allogeneic platforms [2]. However, the transition from experimental success to global commercialization is fundamentally dependent on the ability to preserve these cells without compromising their delicate biological structural and functional integrity. Cryopreservation, the process of cooling biological materials to ultra-low temperatures to arrest metabolic activity, is the enabling technology for this transition [3]. Yet, current evidence indicates that

NK cells are significantly more sensitive to the stresses of freezing and thawing than other lymphoid subsets, frequently manifesting in a post-thaw decline in viability, motility, and cytotoxic potency [3,4].

FUNDAMENTALS OF CRYOBIOLOGICAL INJURY IN NK CELLS

The survival of cryopreserved cells is governed by their physical characteristics, including surface area, volume, and membrane permeability, and their tolerance to the physical and chemical stresses associated with the cryopreservation process [5]. These parameters determine the kinetics of water and cryoprotective agent (CPA) flux across the plasma membrane. Injury primarily occurs during the transition to ultra-low storage temperatures through two competing mechanisms, often described by the 'two factor hypothesis' [6,7]. As a cell suspension cools, the surrounding medium typically remains supercooled until ice nucleation is initiated between -5 °C and -30 °C. Because the cell cytoplasm lacks effective nucleating agents, ice forms first in the extracellular space. This process effectively excludes solutes from the growing ice lattice, significantly increasing the osmolality of the remaining unfrozen fraction. This creates a chemical potential gradient that drives the osmotic dehydration of the cell [6,7]. If the cooling rate is sufficiently slow, the cell remains in osmotic equilibrium with its environment, losing a substantial portion of its intracellular water. However, excessive dehydration leads to lethal 'solution effects' injury [6]. This includes the toxic concentration of intracellular electrolytes, extreme changes in pH, and the mechanical compression of the cell as it shrinks beyond its minimum tolerable volume [8]. Conversely, if the cooling rate is too rapid, the intracellular water cannot exit the cell quickly enough to maintain equilibrium. The cytoplasm becomes increasingly supercooled until it reaches a critical point where intracellular ice formation (IIF) occurs [6,7,9]. IIF is almost universally lethal, as membrane rupture can precede intracellular ice nucleation, and/or the growth of ice crystals within the cell disrupts delicate organelle membranes and the cytoskeleton. Successful cryopreservation requires careful optimization of the stresses encountered by cells during freezing and thawing.

NK cells are particularly sensitive to cryoinjury as they exhibit specific responses to freezing and thawing stressors which differ from other primary immune cells. Investigations using Raman cryomicroscopy and membrane fluidity assays have demonstrated that exposure to cryoprotective agents (CPAs) can reduce the fluidity of the NK cell membrane even before the freezing process begins [10]. This reduction in fluidity impairs the NK cell's ability to maintain its osmotic equilibrium during the subsequent cooling phase. Furthermore, a low membrane permeability to water and cryoprotectants results in the optimal cooling rate for NK cells being approximately 4–5 °C per minute, a rate that is faster than the traditional 1 °C per minute standard used for many other cell types [10]. This suggests that NK cells require a more rapid transition through the critical temperature zone where ice formation and solute concentration are most damaging.

MOLECULAR MECHANISMS OF CRYO-INDUCED DELAYED ONSET CELL DEATH IN NK CELLS

One of the most significant insights into NK cell cryobiology in recent years is the identification of granzyme B (GZMB)-mediated autolysis as a primary driver of the 'delayed' cell death observed after thawing [4]. Unlike T cells, which often show stable post-thaw recovery, NK cells frequently exhibit a phenomenon where viability appears high immediately after rewarming but drops by

as much as 75% within the first 24 hours of culture. High-resolution confocal microscopy and CRISPR-Cas9 gene editing have revealed that the mechanical and osmotic stresses of freezing cause the membranes of cytotoxic granules to become compromised [4]. These granules, which contain the lethal effector proteins GZMB and perforin, are intended to be released into the immunological synapse to destroy target cells. However, when the granule membranes leak during or after cryopreservation, these proteins enter the NK cell's own cytoplasm, initiating a self-destructive apoptotic cascade [4]. This response of NK cell granules to the stress of cryopreservation mimics the same response seen in [11], which exhibit poor functional recovery due to degranulation and low membrane permeability to water and cryoprotectants [5,11,12].

This intrinsic mechanism of damage explains why conventional cryoprotectants often fail to rescue NK cell function even when immediate viability is preserved. The leakage of GZMB triggers both caspase-dependent and independent pathways of programmed cell death [4]. Furthermore, even in cells that do not immediately undergo apoptosis, the loss of motility and the inability to form an effective immunological synapse are frequently reported, likely due to subtle damage to the cytoskeletal architecture and the mobilization of cytolytic granules [13]. Addressing the specific molecular vulnerabilities of NK cells to cryopreservation (Table 1) is now considered a prerequisite for the successful development of off-the-shelf NK cell therapies.

LIMITATIONS OF 'STANDARD' SLOW-COOLING & DMSO PROTOCOLS

The cell therapy industry often adopts a 'one-size-fits-all' approach involving 10% dimethyl sulfoxide (DMSO) and a cooling rate of 1 °C/min [5]. However, this protocol relies on assumptions that do not hold for NK cells. NK cells are notably sensitive to thermal shock and temperature-induced molecular events (Table 1), which can cause damage even before ice formation occurs

DMSO has served as the foundational cryoprotectant for cellular therapies since its discovery in the mid-20th century [14]. As a penetrating CPA, DMSO enters the cytoplasm and disrupts the hydrogen bonding network of water, thereby depressing the freezing point and promoting a vitreous state rather than crystalline ice [15]. Despite its widespread use, DMSO is increasingly viewed as a bottleneck in the development of safer, more potent NK cell therapies [1]. At concentrations typically used for biobanking (5–20%), DMSO exerts significant biochemical toxicity to cells [3]. It has been shown to induce mitochondrial damage in various cell types, alter chromatin conformation, and disrupt the cytoskeleton [14]. In the context of NK cells, the use of DMSO is associated with a reduction in the expression of critical activating receptors such as

TABLE 1

Molecular consequences of cryopreservation on NK cells.

Molecular event	Mechanism of action in NK cells	Consequence for immunotherapy
Granule leakage	Stress-induced permeability of the cytotoxic vesicle membrane	Intracellular release of GZMB and perforin [4]
Autolysis induction	Internal activation of pro-apoptotic signals by leaked granzymes	Massive post-thaw mortality within 24 hours [4]
Cytoskeletal disruption	Interaction of CPAs and ice with actin and tubulin networks	Impaired motility and tumor infiltration [13]
Receptor downregulation	Altered gene expression or membrane shedding post-thaw	Reduced ability to recognize and bind tumor cells [3]

CPA: cryoprotective agent. GZMB: granzyme B.

NKG2D and TRAIL, which are essential for tumor recognition and killing [3].

The clinical implications of using DMSO in clinical cell therapy products continues to be identified as a risk to patient safety. When cryopreserved cell products are infused directly into patients, residual DMSO can trigger a spectrum of adverse reactions, ranging from mild nausea and gastrointestinal distress to severe cardiovascular and neurological events, including cardiac arrest [16]. These risks generally necessitate extensive post-thaw processing, such as washing and dilution, to reduce the CPA concentration to safe levels if there are concerns [16]. However, these additional steps introduce logistical complexity, increase the risk of microbial contamination, and often lead to a further loss of viable cells. Consequently, the cell therapy industry is actively exploring reduced-DMSO and DMSO-free cryoprotectant solution alternatives that can maintain high viability without the associated toxicities [14,17,18].

NOVEL TECHNOLOGIES TO IMPROVE THE CRYOPRESERVATION OF NK CELLS

Pre-freeze and post-thaw molecular modifications

To mitigate the effects of GZMB leakage and general cryogenic stress to NK cells, researchers at the University of Pennsylvania have developed specialized 'rescue' protocols that focus on the biochemical state of the cell prior to freezing. Pretreatment with a combination of Interleukin-15 (IL-15) and Interleukin-18 (IL-18) has emerged as a particularly effective strategy [4]. This cytokine cocktail works through two primary mechanisms. First, it induces a transient state of degranulation, effectively reducing the internal 'toxic load' of GZMB that could potentially leak during the freeze-thaw cycle [4]. Second, it upregulates the expression of anti-apoptotic genes, most notably BCL2L1 (Bcl-XL), which provides the cell with an enhanced internal buffer against apoptotic signals [4]. This approach has been shown to improve post-thaw recovery from 25% to nearly 90–100%, allowing cryopreserved NK cells to perform as effectively as fresh cells in xenograft models of human lymphoma [4].

Beyond direct cytokine stimulation, the metabolic and functional state of the NK cell can be restored through post-thaw 'revitalization' steps. Brief co-culture with effector T cells or the use of synthetic T cells that provide localized IL-2 signaling has been shown to restore motility and cytotoxic function in cryopreserved NK cells, even when assessed in complex 3D environments that better mimic the tumor microenvironment [19]. This finding highlights that the 'ready-to-use' status of an NK cell product is not just a function of its storage conditions, but also of the recovery phase that follows thawing. These strategies are increasingly being integrated into standard operating procedures for the manufacturing of CAR-NK and other advanced cell therapies.

BIOINSPIRED POLYMERS & ICE RECRYSTALLIZATION INHIBITION (IRI) TECHNOLOGY

As alternatives to DMSO are being explored, the field is turning toward bioinspired cryoprotectants that mimic the antifreeze mechanisms found in nature [1,20]. One promising approach involves the use of biocompatible polymers like dextran and carboxylated-poly-L-lysine (PLL) [1]. Unlike DMSO, which primarily acts by reorganizing the hydrogen bonds throughout the solution, these polymers exhibit ice recrystallization inhibition (IRI) activity. Recrystallization, the process where small ice crystals fuse into larger ones during the

warming phase, is often more damaging than the initial freezing process. PLL and similar compounds adsorb to the surface of ice crystals, arresting their growth and maintaining them in a size range that is less harmful to cellular membranes [1,20].

A significant development in this area is the work of companies like PanTHERA CryoSolutions, a BioLife Solutions® company, which has developed novel small carbohydrate-based IRI compounds [20,21]. These inhibitors allow for a substantial reduction in the concentration of traditional CPAs required for stable storage, thereby decreasing biochemical toxicity. Furthermore, IRI technology provides a buffer against transient warming events during transport, which is a major concern for global supply chains that rely on liquid nitrogen or dry ice. By stabilizing the ice-water interface, these bioinspired molecules may function to maintain the integrity of the NK cell membrane and its internal organelles through the entire cryopreservation lifecycle.

COMPARATIVE FREEZING DYNAMICS: SLOW COOLING VERSUS VITRIFICATION IN NK BIOBANKING

The technological choice between slow, controlled-rate freezing (CRF) and vitrification is a subject of intense debate within the cryopreservation community. Controlled-rate freezing, the currently dominant method, uses specialized equipment to lower the temperature in a precise, stepwise manner, usually at 1–5 °C per minute. This method allows for the management of the heat of fusion – the energy released as water transitions to ice – ensuring that the temperature decline remains linear and predictable. CRF is highly scalable and compatible with large-volume cryobags, making it the preferred choice for commercial-scale manufacturing. However, it inevitably involves the formation of extracellular ice, which creates the osmotic and mechanical stressors in NK therapeutic products previously discussed.

In contrast, vitrification involves cooling the sample so rapidly (often >1000 °C per minute) that the water is 'trapped' in an amorphous, glass-like state without ever forming a crystalline lattice [22]. While vitrification eliminates the mechanical damage associated with ice crystals, it typically requires much higher concentrations of CPAs (up to 30–50%) to achieve the necessary viscosity [22,23]. This creates a trade-off between ice-free preservation and chemical toxicity. While vitrification has seen remarkable success in the preservation of oocytes and embryos, its application to trillions of NK cells required for therapeutic doses is limited by the challenges of achieving uniform, ultra-fast cooling in large volumes. Recent evidence suggests that while vitrification may offer superior morphological preservation of tissues, traditional slow freezing with optimized CPA cocktails often yields better functional recovery for singularized immune cells like NK cells [3].

ARTIFICIAL INTELLIGENCE & CRYOBIOLOGICAL OPTIMIZATION

The complexity of NK cell cryopreservation, involving thousands of variables across donor characteristics, media composition, cooling kinetics, and shipping conditions, is increasingly being managed through AI and machine learning (ML). AI is being applied to several critical nodes of the cell therapy lifecycle including the design of new cryoprotectants [24]. Predictive modeling is used to determine the ideal cryoprotectant formulations, and cooling and warming rates for different cell types, reducing the need for costly and time-consuming 'trial and error' experimentation [25,26]. One recent applications of ML in this field is the development of random forest models to predict the cytotoxic efficacy of NK cells based on

their receptor-ligand expression profiles [27]. By analyzing the expression of key pairs, such as NKG2D-MICA/B or KIR-HLA, these models can predict how well a specific batch of cryopreserved NK cells will perform against a patient's tumor with over 84% accuracy [27].

INDUSTRIALIZATION & AUTOMATED WORKFLOWS FOR OFF-THE-SHELF NK THERAPIES

The transition of NK cell therapy from academic centers to centralized manufacturing facilities requires a shift toward automated, closed-system processing [28]. Manual handling of cryopreservation is inherently prone to variability, which can compromise the quality and reproducibility of the final product [29]. Modern automated systems are designed to integrate the harvest, washing, and formulation steps into a single, sterile workflow [28,30]. These systems can resuspend expanded NK cells into a cryopreservation solution with high precision, maintaining cell purity levels above 97% throughout the process [30].

Following formulation, NK cells are transferred to liquid nitrogen-free controlled-rate freezers. Automation also addresses a critical 'bottleneck' identified in industry surveys: the 'window of opportunity' between the addition of the CPA and the initiation of the freeze run [29,31]. In manual processes, this time can vary significantly between batches, leading to inconsistent degrees of CPA penetration and biochemical toxicity. Automated platforms ensure that every vial or cryobag in a batch, which can scale up to 1500 vials or more, undergoes the exact same thermal and chemical experience, ensuring product uniformity.

CONCLUSION & NK CRYOPRESERVATION OUTLOOK

The successful cryopreservation of natural killer cells is not merely a technical requirement; it is the cornerstone of the next generation of cancer immunotherapy. The evidence synthesized in this report highlights a clear shift away from traditional, 'universal' protocols toward specialized, NK-specific strategies that address the cell's unique biophysical and molecular vulnerabilities. The identification of GZMB autolysis as a primary driver of cell death, and the subsequent development of cytokine-based rescue protocols, has already dramatically improved the therapeutic feasibility of cryopreserved NK cells.

As we look toward 2030 and beyond, the convergence of bioinspired chemistry, automated manufacturing, and AI-driven analytics will likely eliminate the 'cryopreservation penalty' currently associated with off-the-shelf products. The industry's move toward reduced DMSO or DMSO-free media, supported by ice recrystallization inhibitors and non-penetrating cryoprotectants, promises to deliver safer and more potent doses to patients. The ultimate success of the field will depend on continued scientific and technical advances, and a relentless focus on the preservation of functional potency, ensuring that every NK cell, once thawed, is ready to fulfill its role as the body's first line of defense against cancer.

REFERENCES

1. El Assal R, Abou-Elkacem L, Tocchio A, *et al*. Bioinspired preservation of natural killer cells for cancer immunotherapy. *Adv. Sci. (Weinh.)* 2019; 6(6), 1802045.

2. Heipertz EL, Zynda ER, Stav-Noraas TE, *et al.* Current perspectives on 'off-the-shelf' allogeneic NK and CAR-NK cell therapies. *Front. Immunol.* 2021; 12, 2021.

3. Saultz JN, Otegbeye F. Optimizing the cryopreservation and post-thaw recovery of natural killer cells is critical for the success of off-the-shelf platforms. *Front. Immunol.* 2023; 14, 2023.
4. Berjis A, Muthumani D, Aguilar O, *et al.* Pretreatment with IL-15 and IL-18 rescues natural killer cells from granzyme B-mediated apoptosis after cryopreservation. *Nat. Commun.* 2024; 15.
5. William N, Sajja A, Acker JP. The cryopreservation of apheresis blood components in cell therapy applications. In: *Apheresis: Principles And Practices* (Editors: Sczepiorkowski Z, Winters JL). 2025; AABB Press, 151–172.
6. Mazur P, Leibo SP, Chu EHY. A two-factor hypothesis of freezing injury: Evidence from Chinese hamster tissue-culture cells. *Exp. Cell Res.* 1972; 71, 345–355.
7. Mazur P. Kinetics of water loss from cells at subzero temperatures and the likelihood of intracellular freezing. *J. Gen. Physiol.* 1963; 47, 347–369.
8. Meryman HT. The exceeding of a minimum tolerable cell volume in hypertonic suspension as a cause of freezing injury. In: *The Frozen Cell* (Editors: Wolstenholme GE, O'Connor M). 1970; Churchill, 51–64.
9. Mazur P. Freezing of living cells: mechanisms and implications. *Am. J. Physiol.* 1984; 247, C125–C142.
10. Joules A, Mallya AS, Louwagie T, Yu G, Hubel A. Probing mechanisms of cryopreservation damage to natural killer cells. *Cytotherapy* 2025; 27(5), 649–660.
11. Arnaud F, Yang H, McGann LE. Freezing injury of granulocytes during slow cooling: role of the granules. *Cryobiology* 1996; 33, 391–403.
12. Yang H, Arnaud F, McGann LE. Cryoinjury in human granulocytes and cytoplasts. *Cryobiology* 1992; 29, 500–510.
13. Oh E, Min B, Li Y, *et al.* Cryopreserved human natural killer cells exhibit potent antitummor efficacy against orthotopic pancreatic cancer through efficicient tumor-homing and cytolytic ability. *Cancers* 2019; 11(7), 966.
14. Ekpo MD, Boafo GF, Xie J, Liu X, Chen C, Tan S. Strategies in developing dimethyl sulfoxide (DMSO)-free cryopreservation protocols for biotherapeutics. *Front. Immunol.* 2022; 13, 2022.
15. Li R, Johnson R, Yu G, McKenna DH, Hubel A. Preservation of cell-based immunotherapies for clinical trials. *Cytotherapy* 2019; 21(9), 943–957.
16. Awan M, Buriak I, Fleck R, *et al.* Dimethyl sulfoxide: a central player since the dawn of cryobiology, is efficacy balanced by toxicity? *Regen. Med.* 2020; 15(3), 1463–1491.
17. Hunt CJ, Johnstone BH, Woods EJ. Barriers to effective cryopreservation of cell therapies: challenges and emerging solution. *Adv. Exp. Med. Biol.* 2025; 1486, 253–282.
18. Yao X, Matosevic S. Cryopreservation of NK and T cells without DMSO for adoptive cell-based immunotherapy. *BioDrugs* 2021; 35(5), 529–545.
19. Zhou X, Zhang S, Yang W, *et al.* Restoring NK cell cytotoxicity post-cryopreservation via synthetic cells. *Adv. Sci. (Weinh.)* 2025; 12(42), e05731.
20. William N, Mangan S, Ben R, Acker JP. Engineering compounds to control ice nucleation and recrystallization. *Annu. Rev. Biomed. Eng.* 2023; 25, 333–362.
21. McMunn LE, Walsh EM, Ben RN. Targeted development and optimization of small-molecule ice recrystallization inhibitors (IRIs) for the cryopreservation of biological systems. *Cryo. Lett.* 2024; 45(2), 69–87.
22. Fahy GM, MacFarlane DR, Angell CA, Meryman HT. Vitrification as an approach to cryopreservation. *Cryobiology* 1984; 21, 407–426.
23. Fahy GM, Levy DI, Ali SE. Some emerging principles underlying the physical properties, biological actions and utility of vitrification solutions. *Cryobiology* 1987; 24, 196–213.
24. Warren MT, Biggs CI, Bissoyi A, Gibson MI, Sosso GC. Data-driven discovery of potent small molecule ice recrystallisation inhibitors. *Nat. Commun.* 2024; 15(1), 8082.
25. Ahmadkhani N, Sugden C, Benson JD, Eroglu A, Higgins AZ. High-throughput evaluation of cryoprotective agents for mixture effects that reduce toxicity. *Cryobiology* 2025; 121, 105316.

26. Ahmadkhani N, Benson JD, Eroglu A, Higgins AZ. High throughput method for simultaneous screening of membrane permeability and toxicity for discovery of new cryoprotective agents. *Sci. Rep.* 2025; 15(1), 1862.

27. Ma J, Yue J, Li Y, *et al.* Machine learning-based development of a cytotoxicity prediction model for NK cell therapy in cancers. *Cell Oncol. (Dordr.)* 2025; 48(6), 1837–1870.

28. Lee S, Joo Y, Lee EJ, *et al.* Successful expansion and cryopreservation of human natural killer cell line NK-92 for clinical manufacturing. *PLoS One* 2024; 19(2), e0294857.

29. International Society for Cell Therapies Cold Chain Management and Logistics Working Group. Cryopreservation – key survey findings from the ISCT Cold Chain Management and Logistics Working Group. 2025. https://www.isctglobal.org/telegrafthub/blogs/ken-ip1/2025/05/28/cryopreservation-key-survey-findings-from-the-isct (accessed Jan 2, 2026).

30. Cytiva. Natural killer cells manufacturing. 2024. https://www.cytivalifesciences.com/en/ca/insights/natural-killer-cells-manufacturing (accessed Jan 2, 2026).

31. Kikuchi T, Takeuchi I, Yamaguchi H. Scalable production process development for NK cells targeting large-scale expansion. *Regen. Ther.* 2025; 30, 535–543.

AFFILIATION

Jason P Acker, Department of Laboratory Medicine and Pathology, University of Alberta; Innovation and Portfolio Management, Canadian Blood Services, Edmonton, AB, Canada

AUTHORSHIP & CONFLICT OF INTEREST

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CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

COMMENTARY

Systemization is key to fulfilling T cell therapy's promise in cancer

Reagan Jarvis

Whilst first generation CAR-T therapies have already transformed cancer treatments, fundamental barriers remain in translating the treatment's success into solid tumors. The development of CAR-T therapies in this space has primarily been impeded by the lack of novel cancer targets and targeting receptors and scalable and cost-effective manufacturing. The development of next generation T cell therapies, like the emerging TCR-T modality, are able to address these barriers to make personalized T cell immunotherapy scalable and accessible for solid tumor patients worldwide. Recent advances in high-throughput screening, genetic engineering, and automated manufacturing now enable systematic, end-to-end development workflows for targeted T cell therapies and it is evident that further innovation across the entire cell therapy development process is essential to transform extraordinary science into sustainable treatments capable of reaching patients everywhere.

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Targeted T cell therapies have already transformed cancer treatment. First generation CAR-T cell therapies work by reprogramming patient T cells to direct these powerful immune cells to recognize and directly eliminate cancerous cells. Today, multiple marketed products have unequivocally delivered life-saving outcomes for previously untreatable hematological cancers.

Yet this success has not translated to solid tumors, which account for more than 90% of cancer diagnoses. The development of CAR-Ts in this space has faced headwinds due to their high cost and challenges in manufacturing scalability. More fundamentally, the CAR-T application to solid tumors has faced major biological barriers including antigen heterogeneity, toxicity, and in my view the most important, inherent targeting limitations.

There are two fundamental barriers that need to be resolved to deliver the transformative impact of targeted T cell therapies to solid tumors:

- Novel cancer targets and targeting receptors; and
- Scalable and cost-effective manufacturing.

The most fundamental barrier to entry for T cell therapies in solid tumors is identification of tumor-selective targets and delivering potent targeting receptors to leverage those cancer targets. CAR-T cell therapies are defined by the synthetic antibody derived targeting receptor, which drives recognition of cell surface antigens in a human leukocyte antigen (HLA) independent manner. To date, marketed CAR-T products has centered on a small number of targets, CD19 and BCMA. These are not cancer-specific antigens, but lineage markers expressed broadly across B cell populations. Their targeting is clinically viable because B cell aplasia is medically manageable. This distinction is critical: the success of CAR-T in hematological malignancies derives not from tumor selectivity, but from the tolerability of collateral damage. In solid tumors, equivalent lineage markers do not exist as the majority of surface antigens are shared between tumor and essential normal tissues.

Tumor selectivity can be achieved through the emerging TCR-T modality, where the targeting receptor deployed is a T cell receptor (TCR), rather than a synthetic CAR receptor. TCR-T therapies harness the natural targeting mechanism of T cell immunity by detecting peptide fragments derived from intracellular proteins presented on the cell surface by HLA molecules. This biology dramatically expands the addressable target space, as oncogenic drivers and tumor-associated aberrations reside intracellularly. Through presentation of peptides derived from mutated or aberrantly expressed proteins, cancer cells become immunologically visible. This TCR/HLA-peptide biology drives the central mechanism of anti-tumor immunity, and TCR unlocks this target space that is inaccessible to CAR-based modalities.

The clinical success of tumor infiltrating lymphocyte (TIL), immune checkpoint inhibitor therapies (ICI), and TCR-T cell therapies center on amplifying, unleashing, and harnessing T cell responses to HLA-peptide targets. However, the vast array of cancer targets, shaped by the wide diversity of HLA genes across the human population, and the extraordinary spectrum of mutated and dysregulated proteins from which the presented tumor-selective peptides are derived, necessitates high levels of personalization in the TCR-T cell therapy approach. In practice, dozens of distinct TCR-T products need to be deployed to meet patient HLA-peptide target diversity in a personalized therapy framework, where patients are eligible for treatment when diagnostically matched for tumor target expression and HLA expression. This complexity has to date constrained TCR-T development and highlights the need for new tools and approaches to enable precise and scalable cancer target selection and T cell receptor generation.

The manufacturing of CAR-T and TCR-T cell therapies share many parallels, with the core objective being to equip patient or donor T cells with the relevant targeting receptor. In less than a decade since the first CAR-T approval, it is estimated that well over $100 billion has been invested into manufacturing technologies and infrastructure, primarily for the CAR-T modality. These investments span vector technologies, gene editing tools, automation platforms, closed-system processing, and digital supply chain infrastructure. Advances span autologous workflows as well as allogeneic donor-derived and induced pluripotent stem cell (iPSC)-based approaches designed to improve scalability and reduce COGs. The more recent emergence of *in vivo* T cell programs seeks to further standardize and reduce costs by essentially making target T cell therapies a gene therapy modality. As a result, there now exists a wealth of manufacturing technologies to deliver targeted cell therapies. However, the clinical success of CAR-T therapies has concentrated commercialization on a handful of viable therapeutic

cancer targets, thus slowing the deployment of these next-generation manufacturing approaches largely due to market forces.

To drive these hard-earned manufacturing innovations into clinical development and towards patients who urgently need new treatments, novel cancer targets, new products, and expansion across new indications is required sooner rather than later. To unlock solid tumors, the field must integrate scalable cancer target and T cell receptor discovery with a scale-out of novel manufactured products. These technologies already exist. What is needed now is systemized deployment to fulfil the promise of personalized T cell therapies for oncology.

END-TO-END SYSTEMIZATION FOR TCR-T DEVELOPMENT

A large number of innovations have been quietly accumulating to address the challenges of HLA-peptide cancer target identification and characterization, as well as development of clinical-quality targeting TCR. These innovations span high-content and high-throughput screening platforms with contemporary molecular genetics and sequencing technologies, and advances in data science tools to support both laboratory implementation and data interpretation. In parallel, new approaches to T cell receptor optimization have emerged to address the challenge of delivering potent and selective TCR against validated therapeutic HLA-peptide targets.

A common thread among the most recent advances has been the focus on precise and scalable analysis of the cellular biology behind HLA-peptide cancer target presentation and TCR activity. The staggering genetic diversity of both the HLA-peptide and TCR systems demand scalable and parallelizable approaches. At the same time, the biological complexity behind this target-receptor interaction between tumor cells and T cells further necessitates the precise analysis of real cell biology outcomes. Technologies for scaled and precise cell analysis platforms are now able to meet the challenge of HLA-peptide target identification and development of associated TCR.

Scalable, systemized development of TCR against defined HLA-peptide targets must be matched by equally scalable and systemized manufacturing of novel TCR-T products for clinical development and commercialization. A wealth of such enabling technologies now exists, many of which are designed with the aim of optimizing the cost of manufactured goods at commercial scale. However, a near-term imperative is the integration of discovery and manufacturing to deliver new first-in-human products at scale. Addressing patient HLA-peptide target diversity requires corresponding diversity of products, and reduction in time and cost to bring new product candidates to humans is the first critical step to towards true scalability of targeted cell therapies in oncology.

Finally, beyond these two fundamental barriers in discovery and manufacturing, many other factors must also be systematically addressed. We are already seeing innovative clinical trial designs emerging, focusing on select indications with high unmet medical need to pursue clear and timely marketing approvals in TCR-T. For cell and gene therapies generally, regulatory frameworks are being established that encourage flexibility in manufacturing approaches in early-stage clinical trials. We are further seeing innovation in rapid quality control and release testing technologies and systems, in addition to digitalization of batch records, logistics, and improved patient management in the clinic.

Together, these developments all highlight the opportunity to systemize the available technologies, tools, and frameworks to scale deployment of TCR-T cell therapies to address a broad array of highly tumor-selective targets that span cancer indications. Standardization of analytical frameworks in discovery is allowing

codification and formalization of both target selection and the generation of potent and safe targeting TCR – while the wealth of manufacturing technologies now permit rapid translation of this new biology to manufactured product candidates for treatment of various cancers. This overall systemization from discovery, manufacturing, and through to clinical development is set to radically expand the applicability of target T cell therapies in oncology.

THE FUTURE

Targeted T cell therapies have shown outsized impact in cancer indications with high unmet medical need. Technology and processes now exist to accelerate the delivery of novel TCR-T products to human trials. New products can now be developed in months rather than years, with costs set to move from tens of millions to only hundreds of thousands of dollars for the development of novel TCR-T products.

We believe that over the next decade, targeted T cell therapies will become cheaper to develop and to manufacture and thus will become more widely accessible through deep coverage of the available HLA-peptide target space. Achieving this will require integration of innovations across the entire discovery and product-development value chain, necessitating the synthesis of standardized discovery, manufacturing, and clinical development to drive systemized development of these therapies. To realize true scalability, we must drive volume across targeted T cell therapy modalities, because, as with any manufactured product, scale reduces costs and expands access.

The promise of T cell therapy has never been in doubt. The opportunity before us is to make these therapies truly scalable and affordable – transforming extraordinary science into a sustainable, global industry capable of reaching patients everywhere.

BIOGRAPHY

Reagan Jarvis is the Co-Founder and CEO of Anocca, a T cell immunotherapy company based out of Sweden, commencing first-in-human clinical trials in 2025 with gene-edited TCR-T cell therapy products in oncology. A New Zealander, Jarvis obtained his PhD in Biochemistry at University of Otago and performed postdoctoral research at the University of Otago and thereafter the German Cancer Research Center (DKFZ) in Heidelberg. He founded Anocca with Mikael Blomqvist in 2014. Anocca currently has a pipeline of over 40 products. It has over 130 staff members, with more than 40 nationalities and operates its own cGMP cell therapy manufacturing site in Södertälje, south of Stockholm

Reagan Jarvis, CEO & Co-Founder, Anocca, Sweden

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

EXPERT INSIGHT

Solid tumors and advanced therapies: why science alone will not deliver the cure

Arnaud Deladeriere

Despite remarkable progress in hematologic cancers, advanced therapies (CAR-T, TCR, *in vivo* CARs, and engineered immune cells) have consistently failed to deliver durable benefit in solid tumors (with the exception of lifileucel and afamitresgene autoleucel). The underlying causes are not purely biological. The tumor microenvironment (TME) is indeed a formidable, multifactorial barrier: a physical and chemical fortress that limits infiltration, perfusion, and persistence. Yet scientific innovation alone cannot solve what is equally a translational and systemic challenge. Capital irrationality, fragmented IP strategies, and the lack of a rational, product-oriented mindset are stalling genuine progress.

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This article explores both the scientific and non-scientific barriers that have defined the 'solid tumor impasse' drawing from two complementary approaches: Neobe's engineered microbial vectors programmed to breakdown physical barriers to infiltration, and KiraGen Bio's multiplex-edited platform, which adds a genetic resilience layer – 'noise-cancelling headphones' enabling programmed cell therapies to maintain durable function in hostile TME – to illustrate how innovation must integrate biology, manufacturability, and commercial realism.

This article reflects an Expert Insight perspective on the evolving challenges of advanced therapies in solid tumors. The companies mentioned are discussed as illustrative examples of broader scientific approaches rather than endorsements of specific platforms. Multiple academic and industrial groups are pursuing diverse strategies to overcome the TME, and which of these approaches ultimately proves successful remains to be determined.

THE MIRAGE OF PROGRESS IN SOLID TUMORS

For over a decade, the cell and gene therapy field has stood at the edge of a paradox. On

one side, a wave of curative responses in hematologic malignancies has validated the transformative potential of CAR-T and related modalities. On the other hand, the same scientific community continues to collide with the brick wall of solid tumors.

It is important to note that progress has not been absent. Recent signals in glioblastoma, GD2-targeted approaches [1], and the US FDA approval of lifileucel (a tumor-infiltrating lymphocyte therapy) for advanced melanoma demonstrate that immune-based therapies can generate meaningful responses in selected solid tumor contexts [2,3]. However, broad and durable efficacy across tumor types remains elusive, underscoring the complexity of the biological and translational barriers involved.

At every major conference, a familiar refrain emerges: 'solid tumors are next'. Yet despite hundreds of preclinical successes and dozens of clinical trials, no advanced therapy has achieved the kind of reproducible, durable efficacy seen in liquid cancers. The reasons often cited (antigen heterogeneity, poor trafficking, and the suppressive TME) are scientifically valid but only partial. The full picture extends beyond biology into how our industry funds, designs, and translates innovation.

The TME is not a single obstacle but a system: an ecosystem of stromal cells, abnormal vasculature, high interstitial pressure, metabolic deprivation, and chronic immunosuppression [4]. Most approaches to date have attacked one layer of this system in isolation, ignoring its interdependence. Meanwhile, non-scientific forces such as irrational capital allocation, regulatory uncertainty, and misaligned incentives for example, have amplified the problem.

To solve the solid tumor challenge, the advanced therapies community must confront an uncomfortable truth: the science is not failing in isolation. The system is.

SCIENTIFIC BARRIERS: THE TUMOR AS AN ECOSYSTEM, NOT A TARGET

The architecture problem

Discussions about the TME are often one-dimensional, focusing on an individual suppressive cell type of choice. Significantly, these discussions often leave out the essential role of the extracellular matrix (ECM) as an omnipresent component of this TME with multifactorial impacts on therapeutic resistance. The ECM is not just a wall surrounding the tumor, as it is often represented – it is more accurately described as a scaffold: a dense, fibrotic, and dynamically remodeling internal architecture that resists infiltration.

In addition to architectural and immunosuppressive factors, other biological hurdles further complicate therapeutic success. Antigen heterogeneity, downregulation, masking, and shedding limit target engagement. Tumor cells can dynamically alter antigen presentation under immune pressure, contributing to resistance. These adaptive mechanisms reinforce the view that the tumor must be understood as a dynamic ecosystem rather than a static target.

Pedro Correa de Sampaio, CEO of Neobe, describes it as "an internal scaffolding, not a wall". This dense ECM structure elevates interstitial fluid pressure, collapses local vasculature, and prevents effective perfusion [5]. As a result, even the most potent therapies (be they antibodies, nanoparticles, or cells) are stranded at the periphery, unable to penetrate the tumor core.

While architectural constraints are widely recognized within the field, therapeutic development has often prioritized modulation of immune signaling over direct modification of the physical TME. In many solid tumors, architectural constraints are

an early and dominant barrier. In others, chemical and metabolic suppression may predominate. In practice, these forces interact, and the relative contribution of each is tumor-type dependent.

Neobe's solution embraces this reality. Their engineered bacterial platform exploits the natural tumor tropism of microbes, deploying them as living agents that are engineered to degrade key ECM components from within. Neobe's technology is anchored on its proprietary biosensor platform – synthetic engineered promoters that respond to the TME, ensuring that this ECM breakdown occurs only in the tumors and does not affect secondary tissues. This *in situ* degradation reduces pressure, restores perfusion, and transforms immune-excluded tumors into more permeable and immunologically responsive tissue. It is a literal re-engineering of the microenvironment, not through force, but through engineered biology.

It is important to note that stromal manipulation is not without controversy. Preclinical studies in pancreatic and breast cancer have demonstrated that complete or uncontrolled stromal depletion can accelerate tumor progression or metastatic spread in certain contexts [6]. These findings have shifted thinking toward controlled remodeling or normalization rather than indiscriminate degradation. Approaches that incorporate tumor-restricted activation or biosensor-driven specificity attempt to mitigate such risks, but the balance between accessibility and containment remains an area of active investigation.

The chemical and functional barriers

In parallel, the TME imposes chronic suppression through cytokines (TGF-β, IL-10), metabolites (adenosine, lactate), hypoxia, and checkpoint signaling (PD-1/PD-L1) – all of which drive infiltrating immune cells toward dysfunction and exhaustion [7].

Aaron Edwards, CEO of KiraGen Bio, likens this to 'background noise', a constant interference that prevents immune cells from hearing the right signals. KiraGen's multiplex base-edited CAR-Ts use a 'noise-cancelling headphone' strategy: six simultaneous edits, made without double-strand breaks, which remove key receptors allowing T cells to sense suppressive cues. Importantly, this approach avoids simply boosting potency (a path that often leads to toxicity) and instead restores intrinsic function by enabling cells to ignore suppression rather than overpower it.

The concept underscores a shift from brute-force immunoactivation toward functional resilience. It recognizes that CAR-Ts already possess the capacity to kill, provided they can persist and operate in the hostile metabolic landscape of a tumor.

Why single solutions fail

The TME's multifactorial nature, architectural and biochemical, means that no single vector, cell type, or gene edit can deliver a cure in isolation. A bacterial degrader cannot sustain anti-tumor immunity alone, and a CAR-T that ignores suppression still requires access to the tumor core. The emerging consensus is that complementarity, not competition, is the way forward.

A practical constraint often underappreciated in conceptual discussions is the engineering burden associated with multiplex strategies. Viral vector payload limits, manufacturing complexity, editing efficiency variability, and regulatory characterization requirements restrict how many functional modifications can realistically be implemented in a single product [8]. Allogeneic platforms further compound this burden by requiring safety edits alongside functional enhancements. Thus, the call for multi-targeted solutions must be

reconciled with technical and manufacturing feasibility, as discussed further below.

Beyond the illustrative examples discussed here, numerous parallel strategies are under active investigation. These include cytokine-armored CAR-T cells, dominant-negative receptor constructs, checkpoint-resistant designs, CAF-targeting approaches, TGF-β traps, oncolytic viral platforms, and combination regimens integrating radiation or checkpoint inhibitors. The field is not static; multi-modal and combination strategies are increasingly emerging. The question is less whether multi-factorial approaches are needed, and more how they can be rationally integrated and translated at scale.

TRANSLATIONAL & MANUFACTURING HURDLES: FROM BENCH TO PRODUCT

Analytical blind spots

In many advanced therapy programs, the challenge of translation lies not in the science but in the analytics. The field has become adept at scaling manufacturing processes yet remains astonishingly weak at defining what a successful product looks like at the molecular or functional level.

For Neobe Tx, the problem is unusually clear: their engineered bacteria are monoseptic by design. They cannot be sterile, as sterility would destroy the therapeutic organism itself. This creates a fundamental conflict with traditional GMP expectations. Instead, their development hinges on sensitive release and potency assays that confirm purity, genetic stability, and ensure their tumor-specific biosensors are not activated during the manufacturing process. This repositioning of the programmable bug as the drug, rather than its active payload, is an analytical paradigm shift that regulatory agencies are still learning to accommodate.

For KiraGen Bio, the complexity is of a different kind. Their CAR-T products carry six base edits, each of which must be characterized without introducing excessive variability. Within a single batch, editing efficiency can vary gene by gene and cell by cell. Traditional product characterization frameworks, built around uniformity, struggle to accommodate this necessary heterogeneity. As Edwards notes, “We optimize for defined composition and function, not monoculture. There is strong evidence that CD4/CD8 interplay and controlled state diversity improve persistence and durability”.

The problem is compounded by the lack of validated potency assays [9]. Viability, the most used metric, correlates poorly with clinical effect. The field is still searching for what truly defines ‘potency’ in a multi-edited, heterogeneous cell product.

Regulatory friction

Both cases highlight the friction between scientific novelty and regulatory precedent. Neobe’s team has worked directly with the FDA to define their CMC path. This early engagement validated their analytical development plan but also underscored how few precedents exist for living bacterial therapeutics. Each assay, from genetic stability to potency, must be justified *de novo* [10].

KiraGen’s case is different but equally instructive. The company deliberately built its process around established CAR-T manufacturing workflows, leveraging known reagents and equipment to minimize translational risk. Yet even with a familiar process, regulators face new questions: How should a multiplex-edited, off-the-shelf CAR-T be qualified? How much functional characterization is ‘enough’?

The regulatory framework is evolving [11], but not fast enough. These innovations reveal an emerging truth: analytical science has become a rate-limiting step in cell therapy translation.

NON-SCIENTIFIC BARRIERS: FUNDING, FRAGMENTATION, & THE MYTH OF THE MOONSHOT

The irrational funding cycle

Perhaps the most insidious barrier to solid tumor progress is not scientific at all. It is financial. As Edwards and Sampaio both observe, venture capital in cell and gene therapy remains dominated by 'moonshot' thinking. Investors chase singular, high-visibility concepts rather than rational, complementary ones.

The case of Dispatch Bio (a company that recently raised over US$200 million) is illustrative. The sheer magnitude of such rounds creates a perception that breakthrough innovation requires $200 million or more in early capital. The result is a self-reinforcing cycle: large funds congregate around a few hyped technologies while smaller, capital-efficient platforms starve.

Pedro Sampaio calls this 'irrational liquidity': capital piling into the same risk corridor, probabilistically guaranteeing future failures and eroding confidence across the sector. When those bets inevitably underperform, investors retreat from the field altogether, starving the very companies that could have succeeded through incremental, rational innovation.

THE ACADEMIC SPIN-OUT TRAP

A related challenge is structural. Most new technologies in oncology originate as academic spinouts centered on a single hypothesis or mechanism. This narrowness, while understandable from an IP perspective, makes it almost impossible to address the TME's multifactorial nature.

As Edwards notes, "People haven't shifted their mindset to comprehensively address more than one thing at a time". The result is a field of brilliant but siloed technologies, each solving one problem perfectly and none solving the overall system.

In this context, collaboration is not a luxury but a necessity. The combination of Neobe's physical-access platform and KiraGen's functional-resilience CAR-Ts illustrates how complementary solutions could, in principle, unlock solid tumors. But achieving such partnerships requires flexible IP frameworks, shared success models, and investors willing to accept that co-ownership of success is better than ownership of failure.

Rethinking clinical strategy: the case for capital-efficient proof

The final non-scientific barrier is the unsustainable cost of proof. In the current US ecosystem, early-stage clinical validation can cost hundreds of millions of dollars, forcing companies into large rounds before they have any human data. This dynamic rewards those who can raise, not those who can deliver.

One emerging strategy to address capital constraints involves initiating first-in-human studies in jurisdictions with established regulatory frameworks for early-phase advanced therapies, such as Australia, the UK, or selected Asian markets. These regions operate under ICH-GCP principles and maintain regulatory oversight but often offer streamlined clinical activation pathways compared to the USA [12]. Importantly, FDA guidance permits the inclusion of well-conducted foreign clinical data in IND submissions under specific conditions [13].

Such sequencing is not regulatory evasion, but rather a capital-efficiency strategy designed to generate early human signal prior to large-scale US trials. Nevertheless, ethical considerations (including patient protection, informed consent, and data transparency) must remain paramount. As more companies explore this path, greater clarity around regulatory reciprocity and global harmonization will be essential.

Such 'capital-efficient trials' could redefine early-stage translation in cell therapy. They are not shortcuts but rational experiments, testing scientific hypotheses at the right scale and cost. If successful, they could mark the beginning of a more sustainable innovation cycle in CGT.

A PATH FORWARD: INTEGRATION OVER INNOVATION

Rational design and the end of the silver bullet

The failures of solid tumor cell therapies share a common thread: overreliance on isolated innovation. The next era must be defined by integration of biology, process, analytics, and strategy.

Rational design begins with a clear understanding of what not to change. The approaches highlighted here exemplify one possible direction for addressing complementary dimensions of the TME. Whether these specific platforms ultimately succeed remains to be determined through clinical validation. However, they illustrate a broader principle: strategies that integrate architectural accessibility with functional resilience may offer a rational path forward in selected tumor contexts.

The role of early CMC thinking

Every innovation is a product only if it can be measured, manufactured, and released. Integrating CMC strategy early (long before IND preparation) is the most practical way to ensure that scientific novelty does not become a translational dead end.

Too many programs still treat manufacturing and analytics as afterthoughts, leading to costly delays and regulatory surprises. The success of solid tumor therapies will depend not only on new science but on new discipline: defining CQAs that matter, validating potency in physiologically relevant systems, and designing scalable, data-rich processes from day 1.

Commercial viability as a scientific discipline

The final shift required is philosophical. Commercial viability must be treated as a design constraint, not an afterthought. Manufacturing cost, scalability, delivery route, and patient selection are not business details, they are part of the biology of translation.

Both Neobe and KiraGen follow these critical principles. Their platforms were conceived not just as scientific innovations but as products that can exist. They use existing infrastructures, realistic trial designs, and modular technologies that can extend to multiple indications. In doing so, they challenge an industry still addicted to technological novelty for its own sake.

TRANSLATION INSIGHT

Solid tumors will not yield to a single innovation but to the integration of many. The biological barriers of the TME (its architecture and chemistry) can be addressed through complementary, rationally designed modalities such as engineered bacteria and multiplex-edited cells. Yet the greater challenge lies outside the lab: aligning capital, regulation, and manufacturing discipline around products that are not just novel but buildable. The next generation of CGT companies must blend scientific ingenuity with commercial realism, designing therapies that can survive not only the TME but the industrial one as well.

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REFERENCES

1. Majzner RG, Ramakrishna S, Yeom KR, *et al.* GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. *Nature* 2022; 603, 934–941.
2. US Food and Drug Administration. FDA grants accelerated approval to lifileucel for advanced melanoma. Feb 16, 2024. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-lifileucel-unresectable-or-metastatic-melanoma.
3. Sarnaik A, Hamid O, Khushalani NI, *et al.* Lifileucel, a tumor-infiltrating lymphocyte therapy in melanoma. *J. Clin. Oncol.* 2021; 39(26), 2972.
4. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. *Cancer Res.* 2019; 79(18), 4557–4566.
5. Stylianopoulos T, Martin JD, Chauhan VP, *et al*. Causes, consequences, and remedies for growth-induced solid stress in tumors. *Proc. Natl Acad. Sci. USA* 2012; 109(38), 15101–15108.
6. Chauhan VP, Boucher Y, Ferrone CR, *et al.* Compression of pancreatic tumor blood vessels by hyaluronan. *Cancer Cell* 2014; 26(1), 14–15.
7. Özdemir BC, Pentcheva-Hoang T, Carstens JL, *et al.* Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 2014; 25(6), 719–734.
8. Milone MC, O'Doherty U. Clinical use of lentiviral vectors. *Leukemia* 2018; 32, 1529–1541.
9. US Food and Drug Administration. Potency tests for cellular and gene therapy products. Jan 2011. https://www.fda.gov/media/79856/download.
10. US Food and Drug Administration. Chemistry, manufacturing, and control (CMC) information for human gene therapy IND applications. Jan 2020. https://www.fda.gov/media/113760/download.
11. US Food and Drug Administration. Potency assurance for cellular and gene therapy products (draft guidance). Dec 2023. https://www.fda.gov/media/175132/download.
12. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Guideline for good clinical practice. Nov 9, 2016. https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf.
13. Electronic Code of Federal Regulations. 21 CFR 312.120 – acceptance of foreign clinical studies. https://www.ecfr.gov/current/title-21/section-312.120.

AFFILIATION

Arnaud Deladeriere PhD, Cell & Gene Consulting Inc., Hamilton, Ontario, Canada

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

COMMENTARY

Role of supportive care in the future of cell therapies

Liam Tremble and Paula Maguire

Supportive care is typically differentiated from disease modifying therapies as its function is not to cure patients of a disease. Supportive care forms the backbone of the healthcare infrastructure and is of pivotal importance in facilitating the delivery of advanced therapies such as cell therapies, which can present challenging tolerability profiles. For instance, cytokine release syndrome or immune effector cell-associated neurotoxicity syndrome, has led to the discontinuation of a number of cell therapies during development.

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Supportive care is a key component of healthcare. It is the bulk of patient's day to day care and has a profound impact on patient quality of life. Despite this, and growing patient advocacy voices amongst clinical research, it has often been neglected by pharma. The slow rate of innovation is highlighted by the predominance of investigator-led rather than company sponsored trials and frequent off-label repositioning of approved medications to compensate for an absence of approved options. Age old medical interventions such as steroids and painkillers still form the backbone of the supportive-care toolkit.

In addition to pharmaceutical interventions, supportive care also incorporates nutritional and practical assistance, palliative and emotional care and physical support, all of which have been shown to improve outcomes [1]. These elements may be of even greater importance with immunotherapies where general health is known to have a profound impact on the immune system, similarly the role of the microbiome is increasingly suspected as a determinant of therapeutic outcome, placing further emphasis on diet and stress [2–4]. Focus on supportive care in prehabilitation and rehabilitation is also vital to deliver advanced therapies at scale. Learnings from stem cell transplant studies are now being applied to the cell therapy setting to reduce symptom burdens [5, 6].

There have been improvements in pharmaceutical options for some areas of supportive care. Pain management, cytopenias, anti-emetics, and anti-allergies have all seen new approvals over recent decades, but there are a large number of underserved

conditions. One example with particular relevance to cell therapies is iatrogenic conditions, which are adverse conditions caused by another medical or surgical intervention. As we enter an era of advanced therapies, we are likely to see an explosion of these conditions. For instance, checkpoint inhibitors emerged around 2011 and effectively gave birth to previously unseen immune-related adverse effects (irAEs) [7]. Checkpoint inhibitors were so profoundly different to previous cancer treatments that clinicians were highly concerned when they saw some tumors rapidly growing, only to learn that this was in fact the tumor swelling with an influx of beneficial cancer-killing immune cells. Clinical use of immunotherapies relied on the development of management strategies for these new irAEs. Similarly, the widespread use of emerging advanced therapies, which are largely still restricted to patients who can access care at large academic teaching hospitals, will be contingent on the development of adequate management strategies.

Emerging cell therapies are producing a growing number of previously unseen therapy associated iatrogenic conditions. Engineered cell therapies may have a risk of malignant transformation. Immunotherapies such as bispecific antibodies (BsAbs) and CAR-T cells have a risk of immune effector cell-associated neurotoxicity syndrome (ICANS) [8]. CRISPR-based therapies may have a risk of off-target genetic modifications [9]. Gene therapies have a risk of genotoxicity related leukemias [10]. CD19 cell therapies induce on-target off-tumor neurotoxicity [11]. Certain immunotherapies induce immune effector cell associated hemophagocytic lymphohistiocytosis (IEC-HLH) [12]. The list of these potentially life-threatening novel conditions is expanding as we see increasingly sophisticated and potent new therapies.

Medics lead the charge by using everything at their disposal to manage patients. Clinical trial experience guides real world use, and extensive examination of supportive care often evades the same level of interrogation that a novel medicine requires. There have been mixed results, some therapies have been discontinued due to the inability to adequately manage their toxicity profile, others have been successfully managed with existing tools but still place onerous requirements on the healthcare system and/or have deleterious effects on the patients. For instance, steroids and tocilizumab, which have been pivotal in managing cytokine release syndrome (CRS) and facilitating the approval of numerous life-saving immunotherapies are highly immunosuppressive and epitomize the repurposing strategy [13]. Given to a population who are already at risk of severe cytopenias and infections, they exacerbate the risk of non-relapse related symptom burden and mortality. Due to the increased infection risk observed with BsAb treatment and CAR-T therapies, infection prophylaxis has been suggested with the UK now offering prophylactic IVIG to all BsAb patients. Prophylaxis with anti-virals such as acyclovir or valacyclovir is also recommended during BsAb treatment for all RRMM patients in addition to SARS-CoV-2 and influenza (with the exception of live formulations) vaccinations.

CRS and ICANS have become consistent adverse effects associated with a growing number of therapies. Both are potentially life-threatening, require prompt intervention and have limited treatment options. Acetaminophen, steroids, and tocilizumab are routinely used for CRS, while steroids are used for ICANS as tocilizumab is not neuro-penetrant. Consensus guidelines are routinely applied with vasopressors and oxygen use guiding CRS grading and cognitive function used to grade ICANS [13]. Use of steroids and tocilizumab have also been used in the setting of prophylaxis to mitigate CRS and ICANS risk [14–16].

Due caution must still be placed on the neutropenia's induced by tocilizumab, and the risks of infection and hyperglycemia

with steroids [17,18]. Additionally, as we have learnt with T cell engaging BsAbs, even among programs which are not outright discontinued, additional measures are still needed such as the use of fractionated step-up dosing to mitigate the severity of CRS, or where possible, by prophylactic disease debulking.

There is a strong medical rationale for the development of a toolkit to facilitate the emergence of the next generation of advanced therapies. Recognizing the immense potential public health benefits they offer, many regulatory agencies have developed frameworks in which to manage these advanced therapies [19]. In the UK these are currently defined as gene therapies, cell therapies, and tissue engineered products, but the list is likely to grow. Their adverse effect profile is likely to be mechanistically predictable and common across classes of emerging products. Without a proactive strategy to medicate these iatrogenic conditions we will fail to realize the potential of these life-saving therapies.

Recently the UKRI have invested in finding much needed solutions by announcing their £10,500,000 prosperity partnerships for advanced therapies safety and toxicity, but significant hurdles remain. In Europe, iatrogenic conditions are typically excluded from the benefits of Orphan Drug Designation despite their rarity [20]. Conducting registrational clinical trials requires access to advanced therapies and for chronic conditions such as cancer this may require prolonged provision of the advanced therapy. Furthermore, regulatory agencies are typically hesitant to provide licenses beyond the clinical trial population in pivotal studies, as these conditions are likely to occur across both drug classes and across indications. They may require complex and challenging clinical trials across different populations, and it is a significant impediment to investment

Repurposing of the existing medical toolkit continues to expose patients to unnecessary risk, and a lack of innovation is exposing patients to a higher level of symptom burden, potentially even compromising the benefit of some breakthrough disease modifying therapies. However, made-for-purpose interventions must travail an uncertain path. Regulatory agencies require robust and statistically significant demonstration of efficacy and a comprehensive safety analysis with no serious unresolved risks. The US FDA's stance to tocilizumab for CRS presents a cautionary tale. Approved for CAR-T cell-induced severe or life-threatening CRS, the FDA preferred to rely on dosing instructions to be provided ad hoc on the label of emerging CRS-inducing treatments and to remain silent on its widespread use for BsAb-induced CRS, an approach that only worked due to the existing licenses of the drug in other indications. Availability of tocilizumab on a named basis was due to a shortage and critical reliance on the product. In many institutions this is still required prior to dosing patients with BsAbs or CAR-T cell therapies. The FDA have provided no dosing instructions for tocilizumab with BsAbs. CRS requires black box warnings, but its management resides in a state of regulatory limbo with no incentives on the horizon to avoid this preventable situation from happening again. The current state is due to legislative and regulatory frameworks in which the FDA require submission of supporting data from a manufacturer to extend a label indication. Where there is generic/biosimilar competition and the relative sales in treating CRS compared to other indications are small, such as in the case of steroids and tocilizumab, a regulatory limbo can emerge. The systematic use of medications in this context must be considered from a regulatory perspective to constitute a serious risk to patient safety.

Although the list of iatrogenic conditions is likely to grow, CRS is a particularly pertinent example as it has a well-defined

pathophysiology and a mature, well accepted grading system. Unlike other inflammatory conditions such as acute respiratory distress syndrome, sepsis, and COVID-19 induced cytokine storm, it is a distinct, well-defined, homogenous condition highly suitable for drug intervention.

Where there is sound and robust evidence, the approved indication of a medication can extend beyond the population included in pivotal trials. This is considered on a case-by-case basis. For example, Anakinra was successful in a pivotal trial in the rare condition of cryopyrin-associated periodic syndromes (CAPS). The trial recruited only those with the most severe form of CAPS known as neonatal-onset multisystem inflammatory disease (NOMID). Although the FDA only granted approval for NOMID, the EMA approved Anakinra for all CAPS patients which includes those with less severe Muckle-Wells syndrome and familial cold autoinflammatory syndrome on the basis of a well-understood pathophysiology in which IL-1 plays a key role and there is a lack of alterative approved options. The regulatory precedents which have been set for CRS tell a more cautionary tale.

The future success of cell therapies is reliant on their safe and effective delivery. Equitable access and scaling beyond the capacity of large academic teaching hospitals requires a multifaceted proactive approach. As we have learnt from the successful effort to support pharmaceutical development for rare and orphan diseases, increased focus on the regulatory precedents and incentives to support the emergence of fit-for-purpose tools to support emerging iatrogenic conditions are critical to ensure the toolkit evolves in parallel with advanced therapies. The road to approved cell therapies has been winding and relied on the alignment of innumerable stakeholders and technologies, it remains to be told if supportive care will be a by-stander or key player in the next generation.

REFERENCES

1. Berman R, Davies A, Cooksley T, *et al.* Supportive Care: an indispensable component of modern oncology. *Clin. Oncol. (R. Coll. Radiol.)* 2020; 32, 781–788.
2. Lasselin J, Alvarez-Salas E, Grigoleit JS. Well-being and immune response: a multisystem perspective. *Curr. Opin. Pharmacol.* 2016; 29, 34–41.
3. Mafe AN, Busselberg D. Microbiome integrity enhances the efficacy and safety of anticancer drug. *Biomedicines* 2025; 13: 422.
4. David A, Lev-Ari S. Targeting the gut microbiome to improve immunotherapy outcomes: a review. *Integr. Cancer Ther.* 2024; 23, 15347354241269870.
5. Obaisi O, Fontillas RC, Patel K, Ngo-Huang A. Rehabilitation Needs for Patients Undergoing CAR-T cell Therapy. *Curr. Oncol. Rep.* 2022; 24, 741–749.
6. McCourt O, Maciocia P, Roddie C, *et al.* Single-centre experience of implementing physiotherapist-led prehabilitation for chimeric antigen receptor T cell therapy. *EJHaem.* 2024; 5, 1033–1037.
7. Lee MR, Dominguez C. MAP kinase p38 inhibitors: clinical results and an intimate look at their interactions with p38alpha protein. *Curr. Med. Chem.* 2005; 12, 2979–2994.
8. Sterner RC, Sterner RM. Immune effector cell associated neurotoxicity syndrome in chimeric antigen receptor-T cell therapy. *Front. Immunol.* 2022; 13, 879608.
9. Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. *Front. Bioeng. Biotechnol.* 2023; 11, 1143157.
10. Benedetti DJ, Marron JM, Kodish E. Gene therapy-related malignancy and the risk of cancer exceptionalism. *J. Clin. Oncol.* 2026; Jco2501844.

11. Gust J, Hay KA, Hanafu LA, *et al.* Endothelial Activation and Blood-Brain Barrier Disruption in Neurotoxicity after Adoptive Immunotherapy with CD19 CAR-T Cells. *Cancer Discov.* 2017; 7, 1404–1419.
12. Hines MR, Knight TE, McNerney KO, *et al.* Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant. Cell Ther.* 2023; 29, 438.e1–438.e16.
13. Lee DW, Santomasso BD, Locke FL, *et al.* ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol. Blood Marrow Transplant* 2019; 25, 625–638.
14. Butman J, Habib A, Welkie RL, *et al.* Steroid Prophylaxis in Patients Receiving CAR-T cell Therapy for Relapsed and Refractory Non-Hodgkin Lymphoma. Blood 2024; 144, 4507–4507.
15. Gavriilaki M, Evangelidis P, Envangelidis N, *et al.* Tocilizumab prophylaxis in patients receiving CAR-T cell immunotherapy for hematological malignancies: a systematic review and meta-analysis. *Blood* 2025; 146, 7668–7668.
16. Kwan A, Sandhu I, Wang P, *et al.* Healthcare resource utilization with prophylactic intravenous anakinra following anti-CD19 CAR-T therapy in B-cell lymphoma: a Canadian single-center quality improvement experience. *Blood* 2025; 146, 2647–2647.
17. Barker HL, Morrison D, Llano A, *et al.* Practical Guide to Glucocorticoid Induced Hyperglycaemia and Diabetes. *Diabetes Ther.* 2023; 14, 937–945.
18. Moots RJ, Sebba A, Rigby W, *et al.* Effect of tocilizumab on neutrophils in adult patients with rheumatoid arthritis: pooled analysis of data from Phase 3 and 4 clinical trials. *Rheumatology (Oxford)* 2017; 56, 541–549.
19. US Food and Drug Administration. *Regulation of Cell and Gene Therapy: A Comprehensive Compliance Framework for CGT Development 2026.* FDA; Dec 2025. https://www.fdaguidelines.com/fda-regulation-of-cell-and-gene-therapy-a-comprehensive-compliance-framework-for-cgt-development-year.
20. O'Connor DJ, Sheean ME, Hofer MP, *et al.* Defining orphan conditions in the context of the European orphan regulation: challenges and evolution. *Nat. Rev. Drug Discov.* 2019; 18, 479–480.

AFFILIATIONS

Liam Tremble PhD, Principal Scientist, Poolbeg Pharma, Dublin, Ireland

Paula Maguire PhD, Scientific Advisor, Poolbeg Pharma, Dublin, Ireland

AUTHORSHIP & CONFLICT OF INTEREST

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CELLULAR IMMUNOTHERAPIES:
TARGETING NEW FRONTIERS

SPOTLIGHT

Leaping forward: how China's biotech evolution is capturing the *in vivo* CAR-T frontier

Zhenghong Gao

INTERVIEW

"Ultimately, the goal remains consistent: addressing large unmet medical needs through whatever combination of global and local capabilities proves most effective."

Yu (Clay) Cao, Vice President, Global Head of Drug Discovery, GenEditBio and Guest Editor of *Cell & Gene Therapy Insights'* February edition speaks with Zhenghong Gao, Co-founder and COO of Uni-Pioneers Bio-Med, Inc., who examines the rise of *in vivo* CAR-T as a transformative modality within China's rapidly evolving biotech ecosystem. The discussion highlights structural differences and complementarities between Chinese and Western innovation models, key technological and manufacturing challenges in delivery platforms, and the role of regulatory agility and evolving funding structures. Together, the conversation offers a forward-looking perspective on how global convergence may shape the next phase of gene and cell therapy innovation.

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Q China biotech has become a major global player in the past few years – from ADCs and bispecific antibodies to cell and gene therapies. In your view, what fundamentally differentiates the Chinese biotech ecosystem from the US or EU in this space?

ZG From my perspective, the Chinese and US/EU biotech ecosystems are structurally different but highly complementary, with each excelling at different stages of innovation and development.

I would characterize China's model as a state-architected system optimized for efficiency and scale. Its greatest strength lies in rapidly and affordably translating established scientific concepts into early-stage clinical proof-of-concept – what is often described as 'fast-follow' innovation. Within this system, I have seen a large number of potential best-in-class drug candidates emerge, supported by coordinated policy, state-linked capital guidance, deep manufacturing capabilities, and a globally trained talent pool.

That said, I do see clear limitations. The ecosystem still lacks sufficient deep risk capital, mature late-stage clinical development expertise, and well-established exit pathways to support truly novel, capital-intensive programs through Phase III trials and global commercialization.

In contrast, I view the US model as a market-driven engine for high-risk, pioneering innovation. It excels at funding and validating 'zero-to-one' science, from discovery through early clinical development, supported by a risk-tolerant venture culture. This system is particularly well-suited for developing first-in-class therapies as proved by Capstan Therapeutics' success of developing a CD19 *in vivo* CAR-T cell therapy for autoimmune disease by using CD8 positive T cell targeted LNP and demonstrated it's early promise in human patients [1–3]. Importantly, it also benefits from a highly mature business development and partnership ecosystem, which plays a critical role in financing late-stage assets. This is further reinforced by payer systems in the US and EU that have historically been more adaptable to high-priced innovative therapies.

Ultimately, I do not see these as different versions of the same system, but rather as models built on fundamentally different foundations – top-down coordination versus decentralized market maturation. They operate most powerfully at opposite ends of the risk and development spectrum. Looking ahead, I believe the future of global biotech may hinge on how these two systems interact, compete, and potentially converge to deliver more innovative and affordable lifesaving medicines to patients worldwide.

Q 2025 has been a breakout year for *in vivo* CAR-T innovation in China. What do you see as the major drivers behind this rapid rise?

ZG At the most fundamental level, long-standing unmet needs in cancer, autoimmune diseases, and other serious conditions are the enduring drivers. In vivo CAR-T represents a highly attractive and rational approach to developing best-in-class – and potentially even first-in-class –therapies that can overcome many of the limitations of ex vivo CAR-T [4].

In my view, the profound challenges of *ex vivo* CAR-T, particularly cost, complexity, and

"I believe the current momentum is real, driven by strong clinical rationale and technical feasibility. However, long-term success will depend entirely on translating early promise into robust, reproducible clinical outcomes."

scalability, have created a strong push for alternative solutions. This has triggered a strategic shift across the industry. Many companies that built mRNA, LNP, and viral vector capabilities during the COVID era are now pivoting toward therapeutic areas with stronger long-term value propositions. *In vivo* CAR-T stands out as an ideal candidate, as it leverages relatively mature delivery platforms to pursue a truly transformative clinical goal, offering a compelling risk –reward profile.

At the same time, advances in delivery technologies, such as LNPs, lentiviral vectors, and AAV, as well as payload engineering, have finally made *in vivo* CAR-T technically plausible. In parallel, multinational pharmaceutical companies are actively seeking this modality to address patent cliffs and maintain therapeutic leadership, creating clear partnership opportunities that help de-risk long-term development for both innovators and investors.

I believe the current momentum is real, driven by strong clinical rationale and technical feasibility. However, long-term success will depend entirely on translating early promise into robust, reproducible clinical outcomes. Beyond the universal challenges of targeting and safety, I see manufacturing and scale-up as the most critical ecosystem-specific bottleneck. I believe the current momentum is real, driven by strong clinical rationale and technical feasibility. However, long-term success will depend entirely on translating early promise into robust, reproducible clinical outcomes.

Q What delivery platforms do you see emerging most prominently for *in vivo* CAR-T in China? Are these approaches more fast-follower in nature, or genuinely innovative? And how do they compare technically with Western peers?

ZG I believe delivery platform innovation will ultimately be decisive for in vivo cell therapy, and the field is far from settled. The core challenge is not delivery per se, but precision delivery. Any viable platform must combine cell-type specificity, low immunogenicity, re-dosability, and scalable manufacturing – an extremely demanding set of requirements.

Given these constraints, I do not believe there will be a single 'one-size-fits-all' solution. Instead, the field is likely to evolve along multiple parallel paths. LNPs, AAV, and lentiviral vectors each have distinct strengths that will likely define different therapeutic niches [5].

For example, I see LNPs, with their potential for lower immunogenicity and repeat dosing, as particularly promising for autoimmune diseases, where safety and chronic administration are critical. Viral vectors such as AAV and lentivirus, with their established ability to drive durable expression, may be better suited for oncology settings where a single, potent intervention could be curative.

Across all platforms, however, scalable manufacturing remains a universal and formidable challenge. Achieving consistent, high-yield, cost-effective production at commercial

scale is a gating factor that many technologies have yet to fully address.

Strategically, I personally place strong emphasis on non-viral delivery, especially next-generation LNPs [6]. Their modularity and scalability align well with the broader goal of making advanced therapies more accessible. What I find most encouraging is the level of innovation in targeting ligands, novel lipid chemistries, and cargo design, all aimed at solving the precision delivery problem. The key next step will be demonstrating robust clinical translatability.

Ultimately, success will belong to platforms that can balance specificity, manufacturability, and, most importantly, therapeutic window [7]. Clinical utility will be the final arbiter: sufficient efficacy with acceptable safety. Identifying the optimal combination of payload, vector, and disease context will be essential to realizing the full promise of *in vivo* cell therapies.

Q There has been extensive discussion around the scale and speed of investigator-initiated trials (IITs) in China. Do you see IITs as a regulatory advantage? And do you anticipate China's genome-engineering guidelines eventually aligning with US FDA or EMA expectations?

ZG I view flexible, well-governed IIT systems as a clear strategic advantage, particularly in early-stage innovation. When embedded within a robust ethical and regulatory framework, IITs allow leading clinical investigators to rapidly test novel concepts and generate early human proof-of-concept data. In my opinion, this agility can be a powerful catalyst for de-risking programs and accelerating development.

Other ecosystems could certainly benefit from studying this model, but its success depends entirely on uncompromising standards for data integrity, patient safety, and scientific rigor.

Regarding regulatory harmonization more broadly, I believe convergence will continue along two parallel dimensions: science and patient-centric mandates. Globally, expectations around safety, quality, and efficacy are increasingly aligned. While procedural differences will remain, the underlying scientific principles guiding *in vivo* genome engineering are moving toward a shared global standard.

Q What funding dynamics are currently powering China's *in vivo* cell therapy boom? And how have recent US – China geopolitical tensions influenced investment trends in genome engineering?

ZG From my perspective, healthcare is a universal imperative that ultimately transcends borders. When I look at funding dynamics, I focus less on nationality and more on how global strengths can be combined to accelerate solutions for patients.

I do observe that cross-border investment remains active, but its form has evolved significantly. Instead of broad, early-stage venture investments, we are now seeing more sophisticated strategic licensing deals and structured partnerships. One increasingly

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common model is the creation of asset-centric 'NewCo' entities, designed to enable shared investment and development under clearly defined terms. This reflects a more focused and calibrated approach to international collaboration.

At the same time, I see several innovative regions pursuing a deliberate balance: building domestic self-sufficiency while selectively opening regulatory and IP frameworks – often through pilot programs in specific hubs – to attract global R&D talent and capital. This is neither a fully open nor closed model, but a nuanced attempt to integrate into global innovation networks on mutually beneficial terms.

Ultimately, the goal remains consistent: addressing large unmet medical needs through whatever combination of global and local capabilities proves most effective.

REFERENCES

1. Hunter TL, Bao Y, Zhang Y, *et al. In vivo* CAR T cell generation to treat cancer and autoimmune disease. *Science* 2025; 388, 1311–1317.
2. BioSpace. Capstan Therapeutics announces initiation of Phase 1 trial of lead *in vivo* CAR-T therapy, CPTX2309, for treating autoimmune disease. 2025. https://www.biospace.com/press-releases/capstan-therapeutics-announces-initiation-of-phase-1-trial-of-lead-in-vivo-car-t-therapy-cptx2309-for-treating-autoimmune-disease.
3. Rapport. Capstan: the story of the open minds behind the acquisition of an *in vivo* CAR-T pioneer. 2025. https://rapport.racap.com/all-stories/capstan-abbvie-car-t-pioneer.
4. Marshall A. *In vivo* CAR-Ts captivate big pharma. *Nat. Biotechnol.* 2025; 43, 1893–1896.
5. Gao Z. Viral and non-viral vector platform evolution. *Cell & Gene Therapy Insights* 2024; 10, 697–699.
6. Gao Z. Exploring the burgeoning role of non-viral approaches in the delivery of nucleic acids. *Cell & Gene Therapy Insights* 2023; 9, 1547–1552.
7. Gao Z. Expanding the therapeutic index of lipid nanoparticles: potential key for clinical translation success. *Cell & Gene Therapy Insights* 2024; 10, 693–696.

BIOGRAPHY

Zhenghong Gao is a scientist and biotech leader pioneering nanotechnologies to transform drug and gene delivery for accelerating drug development. His career spans academia, large pharma, and entrepreneurship, with a focus on engineering smart nanocarriers. His work aims to overcome critical biological barriers in the central nervous system, such as the blood-brain barrier and brain extracellular space, to enable advanced therapies, including gene-editing and *in vivo* cellular reprogramming. These innovative approaches seek to use a patient's own cells as *in situ* factories for producing therapeutics, such as *in vivo* CAR-T.

Dr Gao has authored over 40 publications in top-tier journals such as *Nature Nanotechnology*, *JACS*, and *ACS Nano*, with his research highlighted previously in *Nature* and *Science*, and profile featured by *Cell & Gene Therapy Insights*, selected recipient of national and industry awards. Previously, he served as a Professor at the University of Texas at Dallas (with a cross-appointment at UT Southwestern) and as Head of Nonviral Delivery for Therapeutic Gene Editing at Bayer's Asklepios BioPharmaceutical, Inc. under the leadership of Jude Samulski and Sheila Mikhail. He is now the Co-founder of Uni-Pioneers Bio-Med Inc, where he leads operations and focuses on strategic investments in promising biotech assets, particularly in China.

Zhenghong Gao PhD, Chief Strategy Officer, Zhongmou Therapeutics, North Carolina, USA

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CELLULAR IMMUNOTHERAPIES:
TARGETING NEW FRONTIERS

SPOTLIGHT

The next frontier of *in vivo* CAR-T: a conversation with Shon Green

Shon Green

INTERVIEW

"Future *in vivo* immune cell engineering platforms will deliver not just CAR constructs but additional genetic modifications and programming tailored to enhance potency and safety in the target indication."

Yu (Clay) Cao, Vice President, Global Head of Drug Discovery, GenEditBio and Guest Editor of *Cell & Gene Therapy Insights'* February edition speaks with Shon Green, Cofounder and CSO, Zelig Therapeutics Inc.

In vivo CAR-T therapies promise to revolutionize cell and gene therapy by engineering T cells directly within the body, eliminating the need for labor-intensive and costly *ex vivo* manufacturing. This innovation brings new scientific, operational, and regulatory challenges. In this in-depth interview, Dr Shon Green, a leading scientist and biotechnology executive, shares her perspective on preclinical modeling, first-in-human dosing strategies, CMC hurdles, new therapeutic opportunities, and the evolving global development landscape.

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"When a big pharma company or venture group evaluates an asset, seeing robust NHP proof-of-concept data significantly boosts confidence in translatability."

RETHINKING PRECLINICAL MODELS

Clay: The first question concerns non-human primate (NHP) models. They remain widely used for evaluating *in vivo* CAR-T therapies, but they're also controversial. How do you assess their value and limitations?

Shon: That's a foundational question for any preclinical model. The short answer is that you must match the model to your modality and mechanism of action. The field is actively trying to reduce NHP use, but in some cases – especially with *in vivo* cell engineering and with B cell targeting approaches where POC in NHPs can be meaningful – it's still indispensable.

Viral approaches based on lentiviruses do not translate in most NHP species. Lentiviral integration depends on host factors and most common lab NHP species express TRIM5α which restricts retroviral integration, forcing developers to create surrogate constructs or to use permissive species like pigtail macaques (*Macaca nemestrina*). NHPs might still help answer some aspects of the biology for viral-based approaches, but it depends on your particular drug design.

On the other hand, lipid nanoparticles (LNPs) behave differently: their *in vivo* biodistribution partially depends on serum proteins forming a 'protein corona' around the particle which influences tropism, and this varies between species. It has been shown that there is a poor correlation of LNP tropism between mice and NHPs, so NHPs became essential for predicting LNP tissue distribution in humans.

Clay: For LNPs, the NHP tropism patterns correlate better with humans?

Shon: Exactly. LNP tropism in NHPs is a better predictor of human biodistribution, while mouse data often misleads. For that reason, most developers use NHPs, sometimes early in screening, to understand where their nanoparticles truly go.

That said, NHP data are not usually required by regulators; they are used internally to understand and derisk the biology and to convince investors. When a big pharma company or venture group evaluates an asset, seeing robust NHP proof-of-concept data significantly boosts confidence in translatability.

Clay: And yet, NHP data cannot fully predict human outcomes, right?

Shon: True. There's a famous example – the CD28 superagonist antibody. It was safe in NHPs but caused severe cytokine release syndrome (CRS) in human volunteers. That underscores a key point: NHPs are poor safety models, especially in predicting on-target toxicity. They tolerate higher immune activation without severe clinical symptoms. We use them mainly for efficacy and biodistribution, less for safety.

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Clay: If you see toxicity in NHPs, it is concerning, but an absence of toxicity does not necessarily mean safety?

Shon: That's right. We interpret toxicity as a red flag, but the absence of toxicity does not equal clearance for human trials. You always need large safety margines with your initial dose when moving to first-in-human (FIH) trials.

Clay: Are there viable large-animal alternatives, like dogs?

Shon: Dogs can be useful for biodistribution of some gene-delivery platforms, but they're not good for immune readouts either. Canine T cells do not seem to activate easily, and few reagents cross-react, limiting immunological assessments. They are not typically helpful for predicting immune toxicity or cytokine storm potential.

Clay: Does the optimal approach combine multiple models?

Shon: Yes – integrative modeling is the way forward. Most teams combine rodent, NHP, and *in vitro* systems with *in silico* tools to model dose and exposure. No single system is perfect; multiple models each addressing a specific question are typically combined to assess overall safety and efficacy.

DEFINING THE FIH DOSE

Clay: Moving to dose-finding: how do *in vivo* CAR-T developers determine the FIH dose?

Shon: It's quite different from small molecules or monoclonal antibodies. Those rely on pharmacokinetic modeling and allometric scaling. *In vivo* CAR-T therapy is a living drug. Once a T cell is transduced, it can expand exponentially. Instead of 'dose per kilogram,' we think about functional dose, meaning how many CAR-T cells are generated *in vivo*.

If a similar product exists, the best starting point is benchmarking against prior clinical data. However, if it is a first-in-class product, you calculate from first principles:

- Estimate the number of T cells you need to convert to CAR-T cells for efficacy.
- Determine how many T cells will be exposed to your payload based on route of administration and half-life.
- Measure transduction efficiency per unit of viral particles or LNP.
- Use modeling to predict effective exposure and set the starting dose well below that threshold.

Clay: That sounds highly quantitative.

Shon: It is. We often use mathematical modeling, plus data from *in vitro* titrations and animal exposure to narrow the range, and then apply safety margins of several folds before first dosing in humans.

Clay: You mentioned route of administration. A few drug developers used intranodal injection before moving to intravenous (IV). How do those compare?

Shon: Initially, intranodal administration was appealing, as it confines exposure to a lymphoid environment and possibly activates a uniquely potent subset of T cells. But it's less practical for large-scale clinical use. The whole point of *in vivo* CAR-T engineering is scalability and access – delivering this therapy anywhere, even at community hospitals. Therefore, IV delivery seems more attractive, especially given that preclinical safety/biodistribution profiles are similar.

THE CMC & MANUFACTURING BOTTLENECK

Clay: CMC challenges are notoriously complex in cell and gene therapy. What specific hurdles do *in vivo* CAR-T therapies face?

Shon: Many of the same hurdles as other advanced biologics, but amplified. Three major ones cone to mind: comparability, analytics, and potency testing.

- **Comparability:** Because these are novel and complex biologics and the manufacturing platforms are constantly evolving, it is inevitable that the process will change during development. Any process tweak, for example changing an LNP lipid or changing a purification step, can alter the final product. That triggers re-validation of preclinical data and extends development timelines.

- **Analytics:** Often, novel analytics are required to define your product and characterize its safety and efficacy, and the importance of strong analytics may be underestimated in early development stages. Because these are such complex drugs, we do not understand their critical quality attributes (CQAs) well and therefore need to cast a wide net of analytical characterization to make sure we catch what is important. Without good analytics, drift in the safety or efficacy of your product might go undetected.

- **Potency:** Measuring potency is difficult when your product works by modifying immune cells *in vivo* – you want to characterize how well your product engineers T cells but also must consider the potency of the resulting CAR-T cells. We still lack standardized assays that correlate with clinical response.

Clay: How do you manage that operationally?

Shon: Choose manufacturing partners early and involve them in process development. Many CDMOs are still learning how to handle complex viral or hybrid systems. The selection of CDMO partner is quite important for success and keeping reasonable development timelines. You must actively engage with them and build a real partnership for this to work.

Clay: What about LNP-based systems? Are they easier?

Shon: Untargeted LNPs are simpler: fewer biological components, not requiring a live cell-based system. However, once you start adding targeting ligands – antibodies,

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peptides – you have just added another biologic that needs to be manufactured under cGMP, conjugated to your LNP, and characterized. This increases the analytical burden and COGS which can approach that of viral vectors.

In other words, simplicity is relative; it depends on how you design your LNP.

EXPANDING THERAPEUTIC HORIZONS

Clay: Most *in vivo* CAR-T work started in oncology, but now there is a surge in autoimmune applications. What new indications excite you?

Shon: Autoimmune diseases are the most promising frontier at the moment. Eliminating pathological B cells can revolutionize the treatment of various immune disorders and this application is more straight-forward than in solid tumors where CAR-T cells need a lot of extra enhancements to persist and resist the suppressive TME. It also seems that transient CAR expression can work in this space; you might not need persistence, you just need an immune reset. When you clear B cells deeply enough, new B cells emerge that are not pathological, and patients remain in durable remission even after CAR-T cells vanish.

Clay: And beyond autoimmunity?

Shon: The concept can stretch far. For example:

- Type 1 diabetes: reprogramming effector T cells into regulatory T cells (Tregs) *in vivo.*
- Fibrotic diseases: using transient fibroblast-targeted CARs to remodel tissue safely.
- And my personal area of interest – severe allergies: targeting IgE-producing B cells and plasma cells which is what I am working on right now at Zelig Therapeutics. I believe we can reset the immune system with this approach and basically 'cure' allergies.

Theoretically, any condition where a defined cell population drives pathology, and these cells are not essential for other function or can be re-generated like in the case of B cell variants, could be addressed by *in vivo* immune engineering.

Clay: Recent data suggests that B cell depletion (via CAR-T) even helps in T cell – driven autoimmunity. What is your opinion on this?

Shon: Yes, that's one of those 'happy accidents' in science. We did not predict it, but the results are quite impressive. Eliminating B cells can alter cytokine networks and other immune factors and relieve T cell – mediated inflammation. It is a reminder that immune systems are interconnected in ways we do not fully understand.

Clay: And what about Tregs?

Shon: Tregs are an emerging field with a lot of potential. Broad B cell depletion is powerful but blunt. Tregs offer precision modulation. They can suppress autoimmunity without wiping out entire cell populations. For diseases like chronic graft-versus-host disease

"In the early days, pharma partnerships are mostly financial or symbolic – 'validation' rather than operational support."

or type 1 diabetes, we will likely need both strategies: depletion where necessary, regulation where subtlety counts.

PARTNERSHIPS & THE EVOLVING ECOSYSTEM

Clay: Let's shift to the ecosystem. How do startups navigate partnerships across academia, CDMOs, and big pharma?

Shon: It is about timing and alignment.

- **Academia** is your discovery and translational partner – they bring creativity and mechanistic depth, ideal for early-stage/discovery or correlative studies.
- **CDMOs** provide clinical grade materials and scale, but onboarding takes months. Engage them early; train them well.
- **Pharma** offers financial power and credibility, but their true value arrives in late-stage development and commercialization.

In the early days, pharma partnerships are mostly financial or symbolic – 'validation' rather than operational support. They lend reputation and resources, but real technical integration starts after you show Phase 1 or 2 data.

Clay: Many pharma BD teams seem to prefer big, low-risk deals. Does that discourage early engagement?

Shon: It can, but relationship-building still matters. It may take years for pharma to understand and trust a new platform, so you want to start early. Also, many big pharma companies have venture arms that fund innovation in early stages. Generally, most acquisitions happen once you have strong NHP or human data. Even then, companies start engaging potential pharma buyers months or years before the deal is made so early engagement remains important.

GLOBALIZATION & THE ROLE OF INVESTIGATOR-INITIATED TRIALS

Clay: There is a growing interest in using investigator-initiated trials (IITs) in China to accelerate proof-of-concept. How do you view this shift?

Shon: This is huge. The US risks falling behind if it does not learn from this model. China's IIT ecosystem allows faster, cheaper, and more flexible iteration, not because of lower standards, but because of streamlined structure.

You still need a nonclinical and CMC package that is similar to what is produced for an IND, though specific requirements tend to vary depending on who you engage with. The

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main difference is procedural; hospital ethics committees can approve and launch trials far faster. Patient recruitment is rapid, and investigators are incentivized to publish, so execution is efficient.

A huge advantage of this system is that you can modify designs between cohorts with minimal delay – something nearly impossible under current US IND rules. That speed helps refine design and reach better drug products faster.

China's drug development capabilities are growing fast, and it would serve all of us to take advantage of the speed and cost savings offered by Chinese entities instead of fearing the competition.

THE COMPETITIVE EQUATION & LOOKING AHEAD

Clay: The CAR-T field is crowded. How do small companies stand out?

Shon: Differentiation and timing. Even with similar approaches, being first counts. Intellectual property, market perception, and clinical positioning all lock in early. If you are not first – you need to offer something better. Later entrants need clear advantages, like a novel mechanism, improved efficacy, cleaner safety profile, simpler manufacturing, etc.

Clay: Finally, what do the next five years look like for *in vivo* CAR-T?

Shon: Validation and the first approval. In the next 5 years, we will learn a lot about how integrating and transient *in vivo* CAR engineering approaches work in the clinic and see a surge in additional technologies to enhance efficacy and safety. Future *in vivo* immune cell engineering platforms will deliver not just CAR constructs but additional genetic modifications and programming tailored to enhance potency and safety in the target indication. This evolution will make these therapies more adaptable. Hopefully it will also push *in vivo* CAR engineering into mainstream medicine, not just at specialized centers.

Clay: Any words of caution?

Shon: Be ambitious, but disciplined. This field moves fast, but CMC, data integrity, and patient safety must anchor every decision. The winners will balance creativity with rigor.

BIOGRAPHY

Shon Green trained at UCB and UCSF where she developed and employed preclinical models of cancer to study tumor development and potential therapeutic approaches. She then spent over a decade developing T cell-based immunotherapies for oncology, and more recently autoimmune diseases. Her expertise in cell therapy drug development span autologous CAR- and TCR-engineered T cells, epigenetic and genetic enhancements of T cell function, allogeneic approaches, iPSC-derived products, NK cell engineering, and *in vivo* CAR T cell engineering. Focused on cutting-edge innovations at small to mid-size biotech companies, she guided multiple novel therapeutics through preclinical and CMC development to clinical testing. Currently she consults companies in the cell and gene therapy space while working on launching a new startup biotech that aims to cure severe allergies with *in vivo* CAR technology.

Shon Green PhD, Co-founder and CSO, Zelig Therapeutics Inc., Oakland, CA, USA

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CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

CMC considerations on *in vivo* CAR-T

Dehui Kong

VIEWPOINT

"In the rapidly evolving landscape of *in vivo* CAR-T cell therapies, viral-based approaches, such as those utilizing lentiviral vectors, offer robust integration and persistent CAR expression [...]"

Ex vivo CAR-T therapy requires weeks of manufacturing and release testing, resulting in high costs and prolonged vein-to-vein times. *In vivo* CAR-T eliminates patient-specific processing, making scalable vector production and CMC maturity the key bottleneck. Non-viral mRNA–LNP systems enable rapid, vaccine-like, fully synthetic manufacturing at commercial scale with lower costs, simpler QC, and enhanced safety due to transient expression without genomic integration. In contrast, lentiviral vectors provide durable CAR expression but face complex, expensive production, limited scalability, and extensive safety testing for replication-competent virus and insertional mutagenesis. Non-viral mRNA–LNP platforms currently offer superior manufacturability and faster clinical translation, while viral systems remain preferred when long-term efficacy is essential.

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Conventional *ex vivo* CAR-T manufacturing typically requires ~7–10 days [1,2] for T cell isolation, modification, and expansion. Although next-generation

processes have shortened this to 3 days [3] or even <24 hours [4], mandatory release testing of the final drug product still requires a minimum of 7–14 days. This timeline remains a major barrier to true 'vein-to-vein' speed. It is estimated that a single autologous CAR-T batch currently requires more than 200 labor hours across manufacturing, QA, QC, and supply chain/logistics activities. Labor accounts for approximately 71% of total batch production costs, with manufacturing labor alone contributing up to 48% of the overall expense. In contrast, *in vivo* CAR-T approaches eliminate patient-specific cell processing and associated logistical complexity (apheresis, cryopreservation, centralized manufacturing, and cold-chain distribution). However, they transfer the burden to highly consistent, scalable, and fully characterized delivery vector production. CMC maturity is therefore the rate-limiting step for clinical and commercial success of *in vivo* CAR-T therapies.

Non-viral mRNA–LNP systems are produced using a vaccine-like, fully synthetic process that is rapid, scalable, and cost-effective. Commercial-scale batches (>10–100 g mRNA) are now routine using microfluidic mixing or T-mixers [5,6]. Process analytics and quality control [5,7,8] focus on mRNA integrity, LNP characteristics, and safety attributes. Analytical methods include HPLC, LC-MS, capillary electrophoresis (CE), dynamic light scattering (DLS), ELISA, qPCR, flow cytometry, and so on. Compared to viral-based CAR-T, non-viral mRNA–LNP systems pose fewer safety concerns due to transient transduction, though rigorous testing remains essential. Potency could be assessed via transduction efficiency and T cell killing assays. mRNA–LNP systems offer CMC advantages, including lower production costs, enhanced scalability, and consistent Critical Quality Attributes (CQAs).

Viral-based approaches such as those using lentiviral vector are replication-incompetent but require rigorous control to ensure safety (e.g., no replication-competent lentivirus [RCL]), purity, identity, potency, and stability. CQAs focus on vector titer, genome integrity, impurities, and transduction efficiency, as variations can impact insertional mutagenesis risk, transduction efficacy, and immunogenicity [9,10]. Compared to non-viral CAR-T approaches, viral CMC advantages include a streamlined upstream process and flexibility in CMC modifications, as targeted delivery can be achieved by altering the capsid envelope. However, challenges in scalability, high production costs, vector stability, low titer, and empty capsid content persist, necessitating novel process and analytical development for each new target and capsid modification. Viral and non-viral CMC comparisons are listed in **Table 1.**

To sum up, in the rapidly evolving landscape of *in vivo* CAR-T cell therapies, viral-based approaches, such as those utilizing lentiviral vectors, offer robust integration and persistent CAR expression but grapple with complex manufacturing, high costs, biological impurities like residual host DNA and replication-competent lentivirus, and extensive QC assays including next-generation sequencing (NGS) for insertional sites and biodistribution studies. In contrast, non-viral methods employing mRNA encapsulated in lipid nanoparticles provide a streamlined, vaccine-like production pipeline with scalable microfluidics, lower costs, and simpler chemical purity controls focused on encapsulation efficiency, mRNA integrity, and LNP polydispersity index (PDI), while mitigating integration risks and reducing immunogenicity – though challenges persist in transient expression, off-target liver accumulation, and RNase sensitivity. Ultimately, selecting between these platforms hinges on balancing the need for durable efficacy in oncology applications against manufacturability, safety profiles, and regulatory hurdles under ICH guidelines, with non-viral strategies gaining traction for faster clinical translation in the US and beyond.

TABLE 1

CMC comparisons on viral and non-viral based *in vivo* CAR-T biomanufacturing.

Category	Viral based (e.g. LVV)	Non-viral based (e.g. mRNA-LNP)
Scalability	Difficult beyond ~200–500 L	Difficult beyond 1,000 L
Production cost	High	Low
Batch release timeline	45–90 days	7–14 days
Typical yield/productivity	1×10^{12}–1×10^{14} total TU per batch (low yield); often <20% recovery after purification	5–20 g mRNA per batch at commercial scale; encapsulation efficiency 80–95% achievable
Critical Quality Attributes (CQAs)	Infectious titer (TU/mL)	Particle size and PDI
	Full/empty ratio	Encapsulation efficiency
	Capsid integrity	Ligand density
	Residual host-cell DNA/protein	Residual DNA
	RCL (replication-competent lentivirus)	Potency (*in vitro/in vivo* expression)
Manufacturing risks	Low overall yield	Inconsistent particle formation (size, PDI)
	Batch-to-batch titer variability	Conjugation inconsistency (targeted LNPs)
	Empty capsid contamination	
Analytical risks/challenges	Functional titer	*In vitro–in vivo* correlation of potency
	Full/empty separation analytics	Delivery efficiency
	Transgene integrity in capsid	
Regulatory focuses	RCL testing	Lipid-related toxicity (LNP excipients)
	Biodistribution & persistence	Biodistribution/off-target delivery
	Genotoxicity/integration site analysis	Immunogenicity (repeat dosing concerns)
	Immunogenicity (especially VSV-G)	Residual plasmid DNA impurities
	Long-term follow-up (15+ years for some indications)	*In vivo* expression duration vs prediction from IVIVC

REFERENCES

1. Ayala Ceja M, Khericha M, Harris CM, Puig-Saus C, Chen YY. CAR-T cell manufacturing: major process parameters and next-generation strategies. *J. Exp. Med.* 2024; 221, e20230903.
2. Dias J, Garcia J, Agliardi G, Roddie C. CAR-T cell manufacturing landscape – lessons from the past decade and considerations for early clinical development. *Mol. Ther. Methods Clin. Dev.* 2024; 32, 101250.

3. Svoboda J, Landsburg DJ, Gerson J, *et al*. Enhanced CAR T-cell therapy for lymphoma after previous failure. *N. Engl. J. Med.* 2025; 392, 1824–1835.
4. Yang J, He J, Zhang X, *et al.* Next-day manufacture of a novel anti-CD19 CAR-T therapy for B-cell acute lymphoblastic leukemia: first-in-human clinical study. *Blood Cancer J.* 2022; 12, 104.
5. Hourdel L, Lebaz N, Peral F, *et al.* Overview on LNP-mRNA encapsulation unit operation: mixing technologies, scalability, and influence of formulation & process parameters on physico-chemical characteristics. *Int. J. Pharm.* 2025; 672, 125297.
6. Strelkova Petersen DM, Chaudhary N, Arral ML, Weiss RM, Whitehead KA. The mixing method used to formulate lipid nanoparticles affects mRNA delivery efficacy and organ tropism. *Eur. J. Pharm. Biopharm.* 2023; 192, 126–135.
7. Biavasco R. Evolution of analytical tools and techniques for hematopoietic cell therapy manufacture. *Cell Gene Ther. Insights* 2022; 8, 621–626.
8. Shang D, Zhou Z, Shi R, *et al.* Nanoparticle-based strategy in CAR-T cell immunotherapy: challenges, implications, and perspectives. *Mol. Cancer* 2025; 24, 281.
9. Short L, Holt RA, Cullis PR, Evgin L. Direct *in vivo* CAR-T cell engineering. *Trends Pharmacol. Sci.* 2024; 45, 406–418.
10. Bot A, Scharenberg A, Friedman K, *et al.* *In vivo* chimeric antigen receptor (CAR)-T cell therapy. *Nat. Rev. Drug Discov.* 2025

BIOGRAPHY

Dehui Kong is an Analytical Development Scientist and Quality Control Specialist at UCSF Experimental Cell Therapy GMP facility. She specializes in mathematic modeling, analytical development, CMC quality control for cell and gene therapies, with expertise in CRISPR, CAR-T technologies, flow cytometry, qPCR and ddPCR method development and validation. Prior to joining UCSF, Dehui worked for Stanford University School of Medicine as a postdoctoral researcher and AD scientist, where she contributed to innovative projects in synthetic biology and biosensing. Her research has been published in *Journal of the American Chemical Society* (JACS), *Angewandte Chemie*, *ACS Synthetic Biology*, *ACS Sensors*, *Analytical Chemistry*, and *Synlett*, with over 275 citations on Google Scholar.

Dehui Kong PhD, Analytical Development Scientist, UCSF GMP facility, San Francisco, CA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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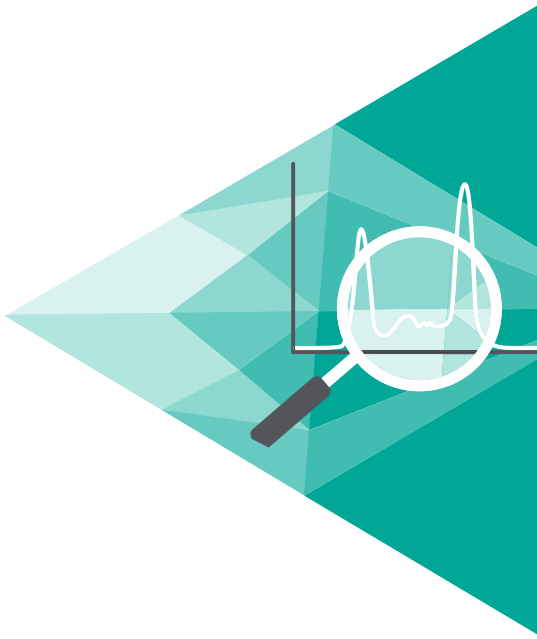
CELL & GENE THERAPY INSIGHTS



ANALYTICS

EXPERT INSIGHT

Integration site analysis in engineered T cell therapies: what we measure, what we miss, and what regulators expect

Deividas Pažėraitis and Juozas Kupčinskas

The safety assessment of genome-engineered cell therapies relies on accurate characterization of vector integration sites, clonal dynamics, and genomic integrity. In CAR-T cell products, integration site analysis (ISA) underpins evaluation of insertional mutagenesis risk, clonal selection, and long-term safety. However, no single assay resolves all relevant dimensions of integration behavior. This article reviews contemporary ISA methodologies through three practical questions: where vectors integrate, how large individual clones become, and which complex genomic events may be missed by standard workflows. We examine the strengths and limitations of restriction-based, fragmentation-based, and unique molecular identifier (UMI)-enabled short-read approaches for population-level mapping and clonal quantification, alongside emerging long-read and orthogonal methods that resolve structural complexity. Emphasis is placed on quantitative biases, structural blind spots, and regulatory interpretation rather than exhaustive method comparison. We propose a layered analytical framework that integrates complementary technologies to support regulatory-grade genomic safety assessment in CAR-T and other engineered T cell therapies.

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TRANSLATION INSIGHT

Integration site analysis should not be treated as a single checkbox assay in CAR-T development. Short-read ISA is highly effective for mapping insertion loci and monitoring clonal dynamics across large populations, but it is inherently limited in resolving structural complexity and atypical integration architectures. Long-read and orthogonal genome-wide methods are therefore not replacements, but targeted audit tools that should be deployed selectively when clonal behavior is unexpected, regulatory questions arise, or complex engineering strategies are

used. From a translational perspective, the critical challenge is not choosing one 'best' method but designing a layered strategy that matches assay resolution to risk. Programs that align ISA depth with development stage, vector design, and observed biology are best positioned to meet regulatory expectations while avoiding unnecessary analytical burden.

INTRODUCTION

Genome-engineered cell therapies are advancing rapidly, driven by improvements in gene editing, delivery technologies, and manufacturing. In terms of industrialization and regulatory maturation, the field increasingly resembles biologics a decade ago: transformative clinical efficacy has been established, while scalability, cost, manufacturing standardization, and long-term safety frameworks continue to evolve. By mid-2025, more than 6,000 interventional cell therapy trials were registered worldwide, over one-third involving genetically modified cells [1]. CAR-T cells have been a flagship modality with Novartis's Kymriah first demonstrating transformative efficacy in B cell leukemia, catalyzing multiple subsequent approvals [2]. Notably, most approved CAR-T products still rely on similar lentiviral vector delivery and CAR designs, reflecting the dominance of lentiviral vector systems due to high transduction efficiency and stable transgene integration [2]. However, contemporary cell therapy platforms increasingly extend beyond single transgene insertion to incorporate deliberate genome editing [3]. Accordingly, genome engineering is now central across these modalities: transgenes are inserted, e.g., CARs or T cell receptors (TCRs), while endogenous genes are disrupted or modified (e.g., *TCR, B2M, PD-1*) to enhance efficacy or safety [3]. This elevates the need for robust precision assessment of genomic modifications to ensure products are both safe and effective.

Off-target nuclease activity and other editing byproducts have been documented in preclinical models, but their incidence in clinical-grade cell products has been substantially mitigated by technological improvements. Engineered CRISPR nucleases and base editors are becoming increasingly specific through strategies such as using 5' truncated guide RNAs (tru-sgRNAs), high-fidelity Cas9 variants (e.g., eSpCas9, SpCas9-HF1, HypaCas9, and 'HiFi' Cas9 R691A), paired nickase approaches, and transient delivery of ribonucleoproteins [4–6]. Base editors and prime editors have similarly incorporated high-fidelity Cas9 domains to minimize off-target effects [4,6]. As a result, small off-target indels or isolated gene knockouts are no longer viewed as the dominant genomic safety concern in cell therapy development – especially since transforming a cell would likely require simultaneous hits in multiple oncogenic pathways, an improbable outcome from controlled editing [7,8].

In contrast, insertional mutagenesis from integrating vectors has a clear clinical precedent for oncogenesis and remains a central genomic safety risk. Natural examples provide a cautionary blueprint: high-risk human papillomavirus (HPV) frequently integrates into the host genome in cervical lesions, with viral insertions correlating with cancer progression [9]. In early gene therapy trials for immunodeficiency, this risk manifested clinically. γ-Retroviral vector–based X-SCID trials in the early 2000s cured immune defects but led to T cell leukemia in several patients due to proviral integration near the *LMO2* proto-oncogene [10,11]. These events established that untargeted integration can dysregulate oncogenes and drive clonal expansion, prompting the adoption of integration site analysis and long-term clonal monitoring as standard safety measures [11]. The subsequent transition to self-inactivating (SIN) lentiviral vectors significantly reduced

genotoxic risk, and hundreds of patients have since been treated without leukemic transformation [12].

However, rare cases of leukemia or lymphoma emerging years after lentiviral gene therapy or CAR-T treatment have been reported [12]. Most notably, in the US FDA-approved lentiviral hematopoietic stem cell (HSC) therapy for cerebral adrenoleukodystrophy (Skysona/elivaldogene autotemcel), over 10% of treated patients developed hematological malignancies, with clonal expansion linked to vector insertions near proto-oncogenes such as *MECOM* and *PRDM16* [13]. These findings underscore that even SIN lentiviral vectors can contribute to oncogenic clonal outgrowth in long-lived stem cell populations. Accordingly, regulatory agencies mandate comprehensive genomic integration analysis and long-term follow-up for gene-modified cell therapies. The FDA and EMA require up to 15 years of patient monitoring for products involving integrating vectors, including periodic assessments for clonal dominance and hematologic abnormalities [11,12]. As the field matures, emerging genome editing approaches aim to avoid random integration altogether. Targeted 'safe harbor' knock-in strategies are under investigation – for instance, inserting a CAR into the TCR α-chain (*TRAC*) locus or the AAVS1 locus on chromosome 19, where transgenes can be expressed without perturbing oncogene/tumor suppressor networks [3].

The clinical success of CAR-T cell therapies in hematological malignancies, together with persistent challenges in scalability, manufacturing complexity, and patient access, has driven the emergence of *in vivo* CAR-T cell generation strategies that are now entering early clinical evaluation. Unlike conventional *ex vivo* manufacturing, these approaches engineer T cells directly within the patient, offering the potential to broaden access while introducing distinct safety considerations associated with genome modification *in situ* [14]. Notably, EsoBiotec's ESO-T01, a nanobody-targeted, immune-shielded lentiviral vector, is the first *in vivo* CAR-T therapy to reach clinical testing. Systemic vector administration enables generation of BCMA-specific CAR-T cells directly in patients with multiple myeloma, eliminating apheresis and centralized manufacturing [15]. Early data show rapid CAR-T expansion with encouraging antitumor activity, providing proof-of-concept for therapeutically effective *in vivo* CAR-T generation [15]. In parallel, non-integrating *in vivo* strategies are being pursued to reduce genotoxic risk. Targeted lipid nanoparticle (LNP) platforms delivering CAR mRNA enable transient, integration-free CAR expression and have now advanced into early clinical testing for rapid, reversible B cell depletion [16]. Collectively, viral and non-viral *in vivo* CAR-T platforms aim to preserve efficacy while fundamentally shifting cell manufacture from the factory to the patient. At the same time, they highlight the need to reassess how insertional mutagenesis risk is evaluated when genome engineering occurs directly *in vivo*. While efficient non-integrating delivery could, in principle, eliminate insertional mutagenesis risk, this remains an aspirational goal; until then, insertional risk remains a central safety consideration for both *ex vivo* and *in vivo* gene-modified cell therapies.

This Technology Insight review examines how insertional mutagenesis risk is evaluated in modern cell therapy programs. We focus on analytical strategies for monitoring transgene integration, assess current integration site and clonal tracking methods, and identify gaps relevant to regulatory-grade genomic safety assessment of *ex vivo* and *in vivo* engineered cell therapies.

HIGH LEVEL OVERVIEW OF REGULATORY EXPECTATIONS

As discussed earlier, insertional mutagenesis is a well-recognized class risk in genome-modified cell therapies, and

regulatory agencies worldwide have implemented strict oversight to address it. Remarkably, the FDA (United States), EMA (Europe), and National Medical Products Administration (NMPA) (China), which collectively oversee >80% of global cell therapy development (based on ClinicalTrials.gov registry data) have converged on nearly identical core requirements. Below is a brief overview of current expectations in these three markets, intended to provide context for the more detailed technical discussion that follows.

HARMONIZED GLOBAL REQUIREMENTS (PRIMARILY FOR *EX VIVO*: EXTENDING TO *IN VIVO* WITH VECTORS)

All three regulatory authorities mandate the following for integrating vector-based cell therapies:

- ISA using validated methods (linear-amplification mediated PCR (LAM-PCR) or ligation-mediated PCR (LM-PCR) with next-generation sequencing (NGS) on each manufacturing lot to confirm polyclonal insertion profiles and absence of dominant or high-risk clones [17–21].
- Vector copy number (VCN) quantification by qPCR or digital PCR, with typical limits of <2 copies per cell serving as lot release criteria [17–21].
- Replication-competent virus (RCV) testing as a mandatory safety release measure [17–21].
- 15-year long-term follow-up (LTFU) with periodic monitoring for vector persistence, clonal expansions, and malignancies [17–21].
- Risk Management Plans (RMPs) explicitly addressing secondary T cell malignancy risk and detailing monitoring strategies for clonal expansions or vector-driven malignancies [21–23].

REGIONAL DISTINCTIONS & RECENT DEVELOPMENTS

United States (FDA)

In April 2024, the FDA introduced a class-wide Boxed Warning for CD19 and BCMA CAR-T products citing risks of secondary T cell malignancies, reinforcing the need for lifelong monitoring and mandating vector integration testing in any new T cell cancers [23]. Further, FDA 2024 draft guidance for allogeneic products recommends whole-genome sequencing at ≥50× coverage for genome-edited or clonally expanded products to assess off-target effects and vector integrations, underscoring the FDA's evolving emphasis on high-resolution genomic characterization across both *ex vivo* and *in vivo* cell therapies [24]. VCN thresholds must be justified by product-specific risk assessments, and if dominant clones emerge or insertions map near oncogenes, further genomic analysis is required [17,18].

Europe (EMA)

Marketing authorization dossiers for CAR-T products must include robust insertional mutagenesis data [21]. For example, the EMA review of Kymriah documented lentiviral insertion-site mapping on 14 batches, showing no enrichment near genes of concern [25]. Emerging pharmacovigilance data have heightened scrutiny: as of early 2024, approximately 38 T cell malignancy cases had been reported post-CAR-T, with EMA concluding a 'reasonable possibility' of causal association in some instances [26]. This prompted revisions to product labels and RMPs, introducing expectations for molecular testing of any post

CAR-T cell malignancy to assess for CAR vector integration [26]. Periodic Safety Update Reports (PSURs) must include any evidence of insertional oncogenesis [21].

China (NMPA)

China's National Medical Products Administration (NMPA) has aligned closely with FDA and EMA expectations for gene-modified cell therapies, issuing CAR-T–specific guidance and long-term follow-up requirements in the early 2020s [19,22]. Chinese IND and NDA submissions are required to address genomic safety through vector characterization, replication-competent virus testing, and integration site profiling, supported by risk management plans covering insertional oncogenesis and long-term clonal risk [19,22]. CAR-T products approved in China are subject to extended post-marketing surveillance, including long-term follow-up and periodic safety reporting focused on gene therapy–specific risks [22].

IN VIVO CELL THERAPIES

While no agency has yet issued a dedicated guidance specific to *in vivo* CAR-T cell generation, the FDA and EMA implicitly apply gene therapy standards to these products. For instance:

- The FDA's 2024 CAR-T and 2020 LTFU guidances apply equally to *in vivo* gene transfer platforms, such as lentiviral or AAV delivery of CAR constructs [18,23].
- Insertional mutagenesis risk assessments, integration site monitoring, and vector biodistribution are expected if the transgene is delivered using an integrating vector (*e.g.*, lentivirus) [17,21].
- Non-integrating *in vivo* platforms (*e.g.*, mRNA–LNPs) may not require ISA or LTFU but still undergo pharmacokinetic and biodistribution evaluations consistent with gene therapy expectations [17,23,27].
- EMA's 2025 ATMP clinical trial guidance requires biodistribution and vector shedding data for any *in vivo*-delivered ATMP, with insertional risk addressed as part of the non-clinical and RMP sections [21].

Accordingly, CAR-T cells generated *in vivo*, integration site analysis, where applicable, shifts from lot-release characterization toward biodistribution-aware, longitudinal monitoring of gene-modified cells and targeted investigation in the event of suspected clonal expansion or malignancy.

DELIVERY PLATFORMS & SAFETY CONSIDERATIONS

CAR-T cell manufacture employs a range of viral and non-viral delivery strategies that differ fundamentally in their genomic risk profile and, consequently, in the scope and depth of insertional safety assessment required. While viral vectors remain dominant in approved products, non-viral approaches are increasingly explored to mitigate integration-associated risks and improve overall safety profiles [28]. Here, delivery platforms are discussed primarily in the context of genome interaction, insertional mutagenesis potential, and downstream analytical implications, rather than transduction efficiency.

Viral vectors remain the most established delivery modality in CAR-T cell therapy. Lentiviral vectors, widely used for clinical CAR-T manufacture, integrate into the host genome and enable durable transgene expression [29–31]. However, stable integration inherently introduces insertional mutagenesis risk, necessitating integration site analysis and

long-term clonal monitoring, as discussed in subsequent sections [29–31]. AAV vectors are generally considered lower risk with predominantly episomal persistence and comparatively mild immune profiles, yet their limited cargo capacity (~4.7 kb) often necessitates dual-vector strategies and high dosing. In some clinical contexts, supraphysiological AAV exposure has been associated with serious toxicities, and pre-existing or induced anti-AAV immunity can preclude re-dosing [32,33]. While AAV integration is infrequent, insertional risk assessment may still be warranted under high-dose or long-term exposure scenarios [32,33].

Non-viral delivery platforms are attractive from a genomic safety perspective, as they largely avoid permanent genome modification and viral protein immunogenicity [33]. LNPs, including mRNA–LNP systems, enable transient expression without genomic integration and have demonstrated clinical feasibility in RNA-based therapies [32,34]. However, transient expression often necessitates higher or repeated dosing, and innate immune activation (e.g., via ionizable lipids or PEG components) remains a practical concern [32,34]. Polymeric nanoparticles offer tunable chemistry and payload flexibility, but face challenges related to manufacturing scalability, biodistribution control, and dose-dependent toxicity [33]. Engineered exosomes provide a biologically derived, low-immunogenic delivery vehicle capable of transporting RNA or protein cargo, yet their low yield and complex purification currently limit clinical deployment [32]. Physical delivery approaches such as electroporation are efficient for *ex vivo* modification but can induce significant cellular stress and reduce product viability [35].

DNA transposon systems, including Sleeping Beauty and piggyBac, occupy an intermediate position between viral and non-viral strategies. These platforms avoid viral components while enabling stable genomic integration and scalable CAR-T manufacturing [36]. However, transposon integration remains semi-random and frequently results in multiple insertions per cell, preserving insertional mutagenesis risk [36]. Integration preferences differ by system – piggyBac favors transcriptionally active regions, whereas Sleeping Beauty exhibits a more dispersed profile – but neither achieves true site specificity [36,37]. As a result, regulatory authorities treat transposon-engineered CAR-T products as integrating gene therapies, subject to the same integration site analysis and clonal safety requirements as lentiviral vector approaches [36].

Emerging *in vivo* CAR-T strategies further highlight the importance of delivery-linked genomic risk. Direct delivery of CAR or gene-editing constructs to patient T cells using viral vectors or nanoparticles bypasses *ex vivo* manufacturing but inherits the safety liabilities of the chosen vector [14,33]. Integrating viral systems raise concerns around insertional oncogenesis and immune responses, while non-integrating approaches such as mRNA–LNPs reduce genomic risk but face challenges in cell-type specificity, efficiency, and cytokine-mediated toxicities if widespread T cell activation occurs [16,33]. Consequently, delivery modality selection directly determines whether insertion site analysis, clonal tracking, or alternative genome integrity assessments are required, reinforcing the need to align analytical strategies with delivery biology (Table 1).

INTEGRATION SITE ANALYSES AND ASSOCIATED METHODS

The safety monitoring of genome-engineered cell therapies relies on detailed analysis of vector integration sites and clonal populations. A variety of technologies have been developed to answer key questions:

TABLE 1

Summary of common gene delivery platforms used in CAR-T and cell therapies.

Platform	Key features/use	Risks/challenges
Lentivirus	Integrating viral vector for durable expression (e.g., CAR-T) [32]	Stable genomic integration confers insertional mutagenesis risk, including oncogene activation; requires integration site analysis, clonal tracking, and long-term follow-up [28–32]
AAV	Small (~4.7 kb) payload; long-term episomal expression; mild immune profile [32]	Predominantly episomal with low reported integration frequency; rare integration events under high-dose or prolonged exposure may warrant targeted integration assessment [38]
LNP	Non-viral carrier for RNA/DNA (e.g., mRNA–LNPs); low immunogenicity, allows repeated dosing [33,34]	No genomic integration; insertional mutagenesis risk is negligible. Genomic safety assessment focuses on biodistribution and persistence rather than ISA [33,34]
Polymeric nanoparticle	Degradable synthetic vectors (e.g., PBAE); tunable targeting, high cargo capacity [33]	Non-integrating delivery minimizes genomic risk; analytical burden centers on biodistribution and off-target exposure rather than insertional analysis [33]
Exosomes	Cell-derived vesicles; very low immunogenicity; multimodal cargo capability (RNA, proteins) [33]	Non-integrating; insertional risk is minimal. Challenges relate to consistency and delivery efficiency rather than genomic safety [33]
Electroporation (*ex vivo*)	Electrical transfection of isolated cells [35]	Efficient *ex vivo* delivery but induces cellular stress and reduced viability; insertional risk is cargo-dependent (RNA vs DNA-based systems) [35]
DNA transposons (Sleeping Beauty, piggyBac)	Non-viral integrating vectors enabling stable CAR expression; cost-efficient and typically delivered by electroporation [36,37]	Semi-random genomic integration with frequent multi-copy insertions; insertional mutagenesis risk comparable to viral vectors; requires full ISA and longitudinal clonal monitoring [36,37]
In vivo CAR T	Direct gene delivery to patient T cells (targeted viral or nano vectors) [33]	Genomic risk depends on delivery modality: integrating vectors pose insertional risk, while non-integrating approaches emphasize biodistribution and persistence [14,33]

ISA: integration site analysis. LNP: lipid nanoparticle.

(1) Where did the vector integrate? (2) How large is each clone? (3) What might we miss? Each question aligns with specific methodological approaches, discussed below. No single assay resolves all dimensions of insertional risk; integration site analysis is therefore best viewed as a layered inference framework rather than a standalone test. Table 2 at the end of this section summarizes these methods, including their input needs, biases, sensitivity, use cases, and regulatory maturity. A comprehensive methodological overview of integration site analysis has been published in November 2025 [11]; here, we focus on genome integration, clonality and emerging technologies.

WHERE DID THE VECTOR INTEGRATE?

Robust mapping of vector integration sites is the first step in insertional mutagenesis assessment. Modern assays typically ligate adapters to genomic DNA and PCR-amplify across the vector–genome junction for NGS-based sequencing. The classic approach is ligation-mediated PCR (LM-PCR or LAM-PCR), which uses restriction enzymes to fragment DNA, extends from the vector long terminal repeat (LTR) with a biotinylated primer, captures products, and ligates linkers for nested PCR [11]. LAM-PCR has high sensitivity (detecting

TABLE 2

Comparison of methods for integration site analysis, clonal assessment, and genome integrity profiling in engineered cell therapies, including input requirements, sensitivity, and typical regulatory use cases.

Method	Input/bias	Sensitivity	Primary use & maturity
LAM-PCR/LM-PCR [41,61]	~0.52 µg DNA (~10^5 cells); restriction enzyme–dependent	Detects clones ≥0.5–1%; blind spots in enzyme-poor regions	Broad mapping of vector insertions (e.g., lentiviral CAR-T). Long-established clinical standard; widely accepted for polyclonality assessment
nrLAM-PCR [40]	~0.52 µg DNA; restriction-free linear amplification	Comparable to LAM-PCR with improved genome coverage	Genome-wide ISA when maximal coverage is required (e.g., HSC gene therapy). Established since ~2009; viewed as enhanced ISA by regulators
Fragmentation-based ISA (sonication/ tagmentation)	≥0.5 µg DNA; random mechanical or transposase fragmentation [51]	Very high; deep NGS enables detection of clones <0.1%	Unbiased global ISA for rare or difficult integrations; increasingly used in translational and clinical settings [39]
UMI-tagged ISA62 (e.g., LUMI-PCR64)	~0.5–2 µg DNA; ISA workflows incorporating UMI-labelled adapters	Similar site recovery with markedly improved quantitative accuracy	Quantitative clonal tracking and longitudinal monitoring; enables molecule-level clone size estimation [52]. Emerging standard with growing regulatory acceptance.
Long-read sequencing (PacBio / Nanopore) [71,74,75]	High-molecular-weight DNA; targeted (e.g., Cas9 capture) or untargeted	Potential single-copy sensitivity; minimal amplification bias; high DNA input	Structural ISA resolving concatemers, rearrangements, and complex insertions. Not routine, but critical for auditing aberrant clones and validating edit integrity
qPCR / ddPCR (VCN assays) [56,60]	Low DNA input; population-averaged; no site resolution	Highly sensitive for total vector burden (ddPCR superior at low copy number)	Average vector copy number for lot release and long-term monitoring. Complementary to ISA; does not resolve integration sites or clonality. Standard regulatory requirement
OGM [76]	≥0.5–1 µg ultra-HMW DNA; PCR-free; SVs ≥5–10 kb	Detects balanced and unbalanced SVs at ~1% allele fraction	Orthogonal genome-wide detection of translocations and large rearrangements invisible to ISA; increasing use as complementary genome integrity assay

ISA: integration site analysis. LAM: linear-amplification mediated. LM: ligation-mediated. NGS: next-generation sequencing. OGM: optical genome mapping. VCN: vector copy number.

insertions down to ~0.1–1% of cells) and has been a mainstay in many trials [11]. However, it suffers from integration-site bias and blind spots: only insertions near chosen restriction sites are amplified, and PCR skews quantitative read counts [11]. In fact, LAM-PCR can miss ~30–40% of clones and distort clone abundance by orders of magnitude without careful controls [39].

An alternative is nonrestrictive LAM-PCR (nrLAM-PCR) [40]. In nrLAM-PCR, genomic DNA is linearly amplified from the vector LTR, linkers are ligated to the single-stranded products, and nested PCR captures junctions [40]. Because no enzyme digest is needed, nrLAM-PCR truly surveys the whole genome. It thus recovers many insertions that standard LAM-PCR misses, especially in enzyme-poor loci [40]. nrLAM-PCR emerged around 2009–2010 and is often run alongside standard LAM-PCR in clinical assays. Its main drawback is modestly lower efficiency on low-input DNA [41], but it is now widely used for regulatory safety profiling of retroviral vectors in hematopoietic cell therapies [42–46].

To reduce these biases, recent protocols replace restriction digest with random DNA fragmentation (sonication or transposase) prior to linker ligation [47–50]. For example, sonication-enabled ISA has mapped tens of thousands of viral insertions in experimental systems and, when coupled with deep sequencing, can detect single-copy integration events [49]. Building on the principle of restriction-free capture, further refinements have focused on simplifying library preparation and reducing DNA input requirements. Tagmentation-based workflows (*e.g.,* DIStinct-seq) compress library preparation to approximately one day and can operate on sub-microgram DNA inputs, substantially improving feasibility for clinical CAR-T sampling, where material is often limited [37,51]. By combining transposase-mediated fragmentation with simultaneous adapter insertion, these approaches reduce hands-on time, DNA loss, and restriction-site bias relative to classical LAM-PCR workflows [51]. Importantly, DIStinct-seq has been shown to recover integration sites and clonal distributions in lentivirally engineered T cells with sensitivity comparable to conventional ISA, while enabling higher throughput and improved reproducibility [51]. As such, tagmentation-based ISA represents a practical evolution of integration site profiling that is well suited for routine CAR-T manufacturing and longitudinal patient monitoring.

Additional gains can be achieved at the bioinformatic level. Refined short-read ISA frameworks, such as MELISSA and INSPIIRED, leverage improved junction filtering, fragment deconvolution, statistical normalization, and standardized visualization to enhance quantitative robustness and reproducibility of fragmentation-based integration site mapping, particularly in heterogeneous clinical samples [52,53]. Lastly, accurate estimation of clone size requires dedicated quantitative approaches that explicitly address PCR amplification bias, which are discussed in the following section.

For known integration loci (e.g., engineered 'knock-in' lines), targeted approaches are used. Targeted Locus Amplification (TLA), for example, cross-links and sequences DNA around a transgene to capture its entire neighborhood [54]. In one gene-therapy cell-bank study, TLA sequencing confirmed vector integrity, identified all integration sites, and accurately counted copy number [55]. Likewise, junction-specific PCR or Sanger sequencing is used to verify expected on-target insertions in genome-edited cells. These targeted assays complement genome-wide ISA by validating the structure of known edits – an important regulatory requirement in precision engineering.

HOW LARGE IS EACH CLONE?

While integration site analysis defines where vectors integrate, assessment of insertional risk ultimately depends on understanding how much each clone contributes to the final cell population and how this distribution evolves over time.

VCN is routinely measured alongside integration site analysis to assess overall genomic burden and support lot-release and long-term follow-up requirements. Quantitative PCR (qPCR) and droplet digital PCR (ddPCR) are the dominant platforms, estimating average vector copies per cell using vector-specific primers normalized to a single-copy host gene [56–59]. qPCR is widely accessible but limited by amplification efficiency, standard curve variability, and reduced accuracy at low copy numbers [60]. ddPCR enables absolute quantification without external standards and provides superior precision and inter-laboratory reproducibility, particularly near regulatory thresholds (e.g., <1–2 copies per cell) [56,59,60]. Critically, both assays provide population-averaged measurements and do not resolve individual integration sites

or clonal architecture [30]. Accordingly, regulators expect VCN assays and integration site analysis to be used together: VCN defines global vector burden, while ISA informs genomic context, clonality, and insertional risk [17,21,22].

Raw read counts from ISA libraries are unreliable proxies for clone size due to PCR bias, fragment-length effects, and stochastic amplification [41,61]. Accurate clonal quantification therefore requires molecular barcoding, in which Unique Molecular Identifiers (UMIs) are incorporated prior to amplification [62]. UMI tagging enables bioinformatic collapse of PCR duplicates, yielding molecule-level counts of vector–genome junctions [62,63]. UMI-based ISA workflows, such as LUMI-PCR, have demonstrated highly reproducible clonal quantification across technical replicates and preserve linearity across a wide dynamic range – from rare (<0.1%) to dominant clones – whereas non-UMI fragment counting approaches plateau at high sequencing depth [64]. When combined with sufficient sequencing depth and appropriate quality controls, UMI-based ISA can report both the identity and relative abundance of each clone within the sampled population [64].

Quantitative clonal data enable detection of clonal selection through analyses such as common insertion site (CIS) testing and longitudinal tracking [12,30,48]. CIS signals must be interpreted cautiously, as large or transcriptionally active genes may accumulate insertions without conferring selective advantage [48]. Regulators therefore expect CIS findings to be supported by clone-size data: true selection is characterized by expansion of one or a few dominant clones rather than numerous low-frequency events [23,40,48]. Longitudinal integration site sequencing remains the most informative approach for identifying emerging clonal dominance [18,21]. Longitudinal profiling integrating ISA with single-cell transcriptomics can directly link clone expansion to transcriptional programs [65] Emerging single-cell approaches now link integration sites to cell state (*e.g.,* chromatin accessibility), enabling clone-level functional annotation rather than abundance alone [66].

In CD19 CAR-T therapy, clinical outcomes have been shown to correlate with genomic modification patterns arising from vector integration [67]. By analyzing patient samples at multiple time points (e.g., baseline, 6mo, 1yr, 5yr), one can plot clonal abundance trajectories. For example, a CAR-T cell clone with a lentivector inserted in the CBL gene was shown to expand markedly over time in a patient treated with anti-CD22 CAR-T therapy, eventually comprising nearly half of circulating lymphocytes as measured by longitudinal quantitative assays [68]. Sustained or progressive expansion, particularly involving cancer-associated genes, constitutes a safety signal warranting investigation [18,23]. Conversely, large but stable clones without pathological features may be considered benign if they plateau and lack secondary genomic alterations [69]. In practice, most gene-therapy products are profiled at infusion and then in long-term follow-up (LTFU) up to 15 years, per regulatory guidance, to catch late-arising expansions [18,21,23].

WHAT MIGHT WE MISS?

Standard integration site analysis (ISA) and clonal tracking workflows used in lentivirally engineered CAR-T cell products are designed to localize insertion sites and estimate relative clone size, but offer limited resolution of the underlying structural architecture of individual integration events [30,47,51]. Short-read ISA approaches, including PCR-free targeted methods such as CReVIS-Seq, enable accurate and multiplexed mapping of lentiviral vector integration sites and clonal

abundance in CAR-T products, making them well suited for population-level screening and monitoring [70], however, when integration is inferred exclusively from short-read junction data, structurally complex events, including concatemers, truncated inserts, local rearrangements or host genomic alterations, may be missed, fragmented, or misinterpreted [47,61,71].

Cross-platform benchmarking demonstrates that long- and short-read integration assays recover overlapping but non-identical classes of events, supporting a complementary 'breadth-and-depth' strategy rather than reliance on a single modality [72]. In practice, lentiviral vectors used in CAR-T manufacturing can integrate as tandem concatemers or in association with local genomic rearrangements [73], scenarios in which short-read methods may detect only outer junctions or underestimate effective VCN.

Long-read sequencing platforms (e.g., PacBio HiFi, Oxford Nanopore) can capture entire integrated lentiviral cassettes together with flanking host DNA in single reads, directly resolving CAR transgene integration architecture. Notably, nanopore sequencing has been deployed as a scalable and cost-effective approach for polyclonal integration site analysis in clinical T cell therapy, supporting feasibility in translational settings [74]. Methods such as pooled CRISPR-inverse PCR sequencing (PCIP-seq) leverage Cas9 cleavage and long reads to recover near-full-length proviral genomes and adjacent host junctions, revealing concatemeric or rearranged integrations that short-read ISA cannot reconstruct [71]. Experience from immune-cell gene therapy has shown that structurally complex integration events, even without overt gene disruption, can drive clonal expansion through altered gene regulation, underscoring the need for full architectural resolution when interpreting CAR-T follow-up data.

PCR-free CRISPR/Cas9 enrichment approaches further extend these capabilities. AFIS-Seq, for example, cleaves internal proviral sequences prior to library preparation, yielding multi-kilobase nanopore reads spanning vector–genome junctions without amplification bias [75]. While powerful, the requirement for high DNA input (~10 µg) currently limits sensitivity in low-VCN or material-constrained CAR-T samples. Finally, although not an insertion site analysis method per se, optical genome mapping (OGM) provides an orthogonal, PCR-free assessment of large-scale chromosomal alterations and large knock-in events that lie outside the resolution of junction-based sequencing and can therefore complement ISA when broader genome integrity questions arise [76].

INTEGRATED PERSPECTIVE

Comprehensive insertional safety assessment in engineered T cell therapies requires a layered analytical strategy. Short-read integration site analysis provides sensitive, scalable mapping of insertion loci across large CAR-T populations. UMI-enabled quantification and longitudinal profiling resolve clonal contribution and selection dynamics. Long-read and orthogonal genome-wide methods address structural blind spots, revealing complex integration architectures and large-scale genomic alterations invisible to junction-based assays. Together, these complementary approaches form a coherent framework that supports regulatory-grade evaluation of genomic integrity, clonal behavior, and long-term safety in CAR-T cell products.

REFERENCES

1. Benthani F, Upadhaya S, Zhou A. Cancer cell therapies: global clinical trial trends and emerging directions. *Nat. Rev. Drug Discov.* 2025; 24, 898–899.
2. Qian Y, Ma W, Xu XN. Breakthrough for anticancer immunotherapy: current advances in manufacturing protocols of chimeric antigen receptor-based therapies. *Antibodies* 2025; 14, 105.
3. Zhang S, Wang Y, Mao D, *et al.* Current trends of clinical trials involving CRISPR/Cas systems. *Front. Med.* 2023; 10, 1292452.
4. Tao J, Bauer DE, Chiarle R. Assessing and advancing the safety of CRISPR-Cas tools: from DNA to RNA editing. *Nat. Commun.* 2023; 14, 212.
5. Kleinstiver BP, Pattanayak V, Prew MS, *et al.* High-fidelity CRISPR – Cas9 nucleases with no detectable genome-wide off-target effects. *Nature* 2016; 529, 490–495.
6. Naeem M, Majeed S, Hoque MZ, Ahmad I. Latest developed strategies to minimize the off-target effects in CRISPR-Cas-mediated genome editing. *Cells* 2020; 9, 1608.
7. Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. *Front. Bioeng. Biotechnol.* 2023; 11, 1143157.
8. Kalter N, Fuster-García C, Silva A, *et al.* Off-target effects in CRISPR-Cas genome editing for human therapeutics: progress and challenges. *Mol. Ther. Nucleic Acids.* 2025; 36, 102636.
9. Shulzhenko N, Lyng H, Sanson GF, Morgun A. Ménage à trois: an evolutionary interplay between human papillomavirus, a tumor, and a woman. *Trends Microbiol.* 2014; 22, 345–353.
10. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, *et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; 302, 415–419.
11. Kochergin-Nikitsky K, Lavrov A, Smirnikhina S. Methodological landscape in the field of integration site identification of retroviruses and retroviral vectors. *Front. Bioeng. Biotechnol.* 2025; 13, 1708724.
12. Ottaviano G, Qasim W. Current landscape of vector safety and genotoxicity after hematopoietic stem or immune cell gene therapy. *Leukemia* 2025; 39, 1325–1333.
13. Cancer cases in bluebird's gene therapy trials. *Nat. Biotechnol.* 2024; 42, 1626.
14. Bot A, Scharenberg A, Friedman K, *et al. In vivo* chimeric antigen receptor (CAR)-T cell therapy. *Nat. Rev. Drug Discov.* 2025; 1–22.
15. Xu J, Liu L, Parone P, *et al. In-vivo* B cell maturation antigen CAR T cell therapy for relapsed or refractory multiple myeloma. *Lancet* 2025; 406, 228–231.
16. Billingsley MM, Gong N, Mukalel AJ, *et al. In vivo* mRNA CAR T cell engineering via targeted ionizable lipid nanoparticles with extrahepatic tropism. *Small* 2023; 20, e2304378.
17. US Food and Drug Administration. *Postapproval Methods to Capture Safety and Efficacy Data for Cell and Gene Therapy Products.* 2025; FDA. https://www.regulations.gov/docket/FDA-2025-D-3049.
18. US Food and Drug Administration. *Long Term Follow-up after Administration of Human Gene Therapy Products.* 2020; FDA. https://www.regulations.gov/docket/FDA-2018-D-2173.
19. Center for Drug Evaluation. *Technical Guidelines For Long-Term Follow-Up Clinical Studies Of Gene Therapy Products.* 2021; CDE. https://www.cde.org.cn/main/news/viewInfoCommon/c9de887410ddcc291ce5a1c039a241c6
20. US Food and Drug Administration. *Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus during Product Manufacture and Patient Follow-up.* 2020; FDA. https://www.regulations.gov/docket/FDA-1999-D-0081
21. European Medicines Agency. *Guideline on Quality, Non-Clinical and Clinical Requirements for Investigational Advanced Therapy Medicinal Products in Clinical Trials.* 2025; EMA.
22. Center for Drug Evaluation.*Technical Guidelines For Clinical Risk Management Plan Of Chimeric Antigen Receptor T Cell (CAR-T) Therapeutic Products.* 2022; CDE.

23. US Food and Drug Administration. *Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products.* 2024; FDA. https://www.regulations.gov/docket/FDA-2021-D-0404.
24. US Food and Drug Administration. *Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products.* 2024; FDA.
25. European Medicines Agency. Assessment report: Kymriah. 2018; EMA.
26. Berg P, Bakker C, Sander M, *et al.* Secondary malignancy of T cell origin after CAR T cell therapy: EMA's conclusions from the evaluation of 38 suspected cases. *Gene Ther.* 2025; 1–6.
27. Cai Y, Sui L, Wang J, *et al.* Post-marketing surveillance framework of cell and gene therapy products in the European Union, the United States, Japan, South Korea and China: a comparative study. *BMC Med.* 2024; 22, 421.
28. Yin H, Wei X. The design of retroviral vectors used in the CAR-T products, risk management, and future perspective. *MedComm.* 2025; 6, e70067.
29. Vargas JE, Chicaybam L, Stein RT, *et al.* Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives. *J. Transl. Med.* 2016; 14, 288.
30. Shao L, Shi R, Zhao Y, *et al.* Genome-wide profiling of retroviral DNA integration and its effect on clinical pre-infusion CAR T cell products. *J. Transl. Med.* 2022; 20, 514.
31. Ciuffi A. Mechanisms governing lentivirus integration site selection. *Curr. Gene Ther.* 2008; 8, 419–429.
32. Geng G, Xu Y, Hu z, *et al.* Viral and non-viral vectors in gene therapy: current state and clinical perspectives. *eBioMedicine* 2025; 118, 105834.
33. Huang Y, Cao R, Wang S, *et al. In vivo* CAR-T cell therapy: new breakthroughs for cell-based tumor immunotherapy. *Hum. Vaccines Immunother.* 2025; 21, 2558403.
34. Kenjo E, Hozumi H, Makita Y, *et al.* Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice. *Nat. Commun.* 2021; 12, 7101.
35. Napotnik TB, Polajžer T, Miklavčič D. Cell death due to electroporation – a review. *Bioelectrochemistry* 2021; 141, 107871.
36. Magnani CF, Tettamanti S, Alberti G, *et al.* Transposon-based CAR T cells in acute leukemias: where are we going? *Cells* 2020; 9, 1337.
37. Hamada M, Nishio N, Okuno Y, *et al.* Integration mapping of piggyBac-mediated CD19 chimeric antigen receptor T cells analyzed by novel tagmentation-assisted PCR. *EBioMedicine* 2018; 34, 18–26.
38. Ertl HCJ. Immunogenicity and toxicity of AAV gene therapy. *Front. Immunol.* 2022; 13, 975803.
39. Harkey MA, Kaul R, Jacobs MA, *et al.* Multiarm high-throughput integration site detection: limitations of LAM-PCR technology and optimization for clonal analysis. *Stem Cells Dev.* 2007; 16, 381–392.
40. Paruzynski A, Arens A, Gabriel R, *et al.* Genome-wide high-throughput integrome analyses by nrLAM-PCR and next-generation sequencing. *Nat. Protoc.* 2010; 5, 1379–1395.
41. Gabriel R, Kutschera I, Bartholomae CC, Kalle C von, Schmidt M. Linear amplification mediated PCR – localization of genetic elements and characterization of unknown flanking DNA. *J. Vis. Exp.* 2014; e51543.
42. Chin CJ, Cooper AR, Lill GR, *et al.* Genetic tagging during human mesoderm differentiation reveals tripotent lateral plate mesodermal progenitors. *Stem Cells* 2016; 34, 1239–1250.
43. Shi Q, Kuether KL, Chen Y, *et al.* Platelet gene therapy corrects the hemophilic phenotype in immunocompromised hemophilia A mice transplanted with genetically manipulated human cord blood stem cells. *Blood* 2014; 123, 395–403.
44. Carbonaro DA, Zhang L, Jin X, *et al.* Preclinical demonstration of lentiviral vector-mediated correction of immunological and metabolic abnormalities in models of adenosine deaminase deficiency. *Mol. Ther.* 2013; 22, 607–622.
45. Kok YL, Vongrad V, Chaudron SE, *et al.* HIV-1 integration sites in CD4+ T cells during primary, chronic, and late presentation of HIV-1 infection. *JCI Insight* 2021; 6, e143940.

46. Marh J, Stoytcheva Z, Urschitz J, *et al.* Hyperactive self-inactivating piggyBac for transposase-enhanced pronuclear microinjection transgenesis. *Proc. Natl Acad. Sci.* 2012; 109, 19184–19189.

47. Oziolor EM, Kumpf SW, Qian J, *et al.* Comparing molecular and computational approaches for detecting viral integration of AAV gene therapy constructs. *Mol. Ther. Methods Clin. Dev.* 2023; 29, 395–405.

48. Hacein-Bey-Abina S, Garrigue A, Wang GP, *et al.* Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Investig.* 2008; 118, 3132–3142.

49. Justice JF, Morgan RW, Beemon KL. Common viral integration sites identified in avian leukosis virus-induced B cell lymphomas. *mBio.* 2015; 6, e01863–15.

50. Wells DW, Guo S, Shao W, *et al.* An analytical pipeline for identifying and mapping the integration sites of HIV and other retroviruses. *BMC Genom.* 2020; 21, 216.

51. Kim J, Park M, Baek G, *et al.* Tagmentation-based analysis reveals the clonal behavior of CAR-T cells in association with lentivector integration sites. *Mol. Ther. Oncolytics.* 2023; 30, 1–13.

52. Lin TY, Ceoldo G, House K, *et al.* Modeling integration site data for safety assessment with MELISSA. *Nat. Commun.* 2025; 16, 7868.

53. Sherman E, Nobles C, Berry CB, *et al.* INSPIIRED: a pipeline for quantitative analysis of sites of new DNA integration in cellular genomes. *Mol. Ther. Methods Clin. Dev.* 2016; 4, 39–49.

54. Vree PJP de, Wit E, Yilmaz M, *et al.* Targeted sequencing by proximity ligation for comprehensive variant detection and local haplotyping. *Nat. Biotechnol.* 2014; 32, 1019–1025.

55. Singh P, La NI, Terrao M, *et al.* Enhancing lentiviral production for WAS gene therapy: a comparative analysis of stable producer cell lines evaluating flatware system and adherent bioreactors in perfusion mode. *Front. Bioeng. Biotechnol.* 2025; 13, 1648028.

56. Parcelier A, Buisset J, Charrier S, *et al.* VCN analysis using droplet digital PCR method in hematopoietic stem cells and T lymphocytes after lentiviral transduction: optimization and limitations. *ReGEN Open* 2022; 2, 28–36.

57. Milone MC, Fish JD, Carpenito C, *et al.* Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy *in vivo*. *Mol. Ther.* 2009; 17, 1453–1464.

58. Biffi A, Montini E, Lorioli L, *et al.* Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science* 2013; 341, 1233158.

59. Woon ST, Mayes J, Quach A, *et al.* Droplet digital PCR for identifying copy number variations in patients with primary immunodeficiency disorders. *Clin. Exp. Immunol.* 2021; 207, 329–335.

60. Kandell J, Milian S, Snyder R, Lakshmipathy U. Universal ddPCR-based assay for the determination of lentivirus infectious titer and lenti-modified cell vector copy number. *Mol. Ther. Methods Clin. Dev.* 2023; 31, 101120.

61. Gabriel R, Eckenberg R, Paruzynski A, *et al.* Comprehensive genomic access to vector integration in clinical gene therapy. *Nat. Med.* 2009; 15, 1431–1436.

62. Kivioja T, Vähärautio A, Karlsson K, *et al.* Counting absolute numbers of molecules using unique molecular identifiers. *Nat. Methods.* 2011; 9, 72–74.

63. Yan A, Baricordi C, Nguyen Q, *et al.* IS-Seq: a bioinformatics pipeline for integration sites analysis with comprehensive abundance quantification methods. *BMC Bioinform.* 2023; 24, 286.

64. Dawes JC, Webster P, Iadarola B, *et al.* LUMI-PCR: an Illumina platform ligation-mediated PCR protocol for integration site cloning, provides molecular quantitation of integration sites. *Mob. DNA.* 2020; 11, 7.

65. Sheih A, Voillet V, Hanafi LA, *et al.* Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. *Nat. Commun.* 2020; 11, 219.

66. Wang W, Fasolino M, Cattau B, *et al.* Joint profiling of chromatin accessibility and CAR-T integration site analysis at population and single-cell levels. *Proc. Natl. Acad. Sci.* 2020; 117, 5442–5452.

67. Nobles CL, Sherill-Mix S, Everett JK, *et al.* CD19-targeting CAR T cell immunotherapy outcomes correlate with genomic modification by vector integration. *J. Clin. Investig.* 2019; 130, 673–685.

68. Shah NN, Qin H, Yates B, *et al.* Clonal expansion of CAR T cells harboring lentivector integration in the CBL gene following anti-CD22 CAR T cell therapy. *Blood Adv.* 2019; 3, 2317–2322.

69. Cavazzana-Calvo M, Payen E, Negre O, *et al.* Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. *Nature* 2010; 467, 318–322.

70. Kim HS, Hwang GH, Lee HK, *et al.* CReVIS-Seq: a highly accurate and multiplexable method for genome-wide mapping of lentiviral integration sites. *Mol. Ther. Methods Clin. Dev.* 2021; 20, 792–800.

71. Artesi M, Hahaut V, Cole B, *et al.* PCIP-seq: simultaneous sequencing of integrated viral genomes and their insertion sites with long reads. *Genome Biol.* 2021; 22, 97.

72. Sheehan M, Kumpf ST, Qian J, *et al.* Comparison and cross-validation of long-read and short-read target-enrichment sequencing methods to assess AAV vector integration into host genome. *Mol. Ther. Methods Clin. Dev.* 2024; 32, 101352.

73. Luqman MW, Jenjaroenpun P, Spathos J, *et al.* Long read sequencing reveals transgene concatemerization and vector sequences integration following AAV-driven electroporation of CRISPR RNP complexes in mouse zygotes. *Front. Genome Ed.* 2025; 7, 1582097.

74. Zhang P, Ganesamoorthy D, Nguyen SH, *et al.* Nanopore sequencing as a scalable, cost-effective platform for analyzing polyclonal vector integration sites following clinical T cell therapy. *J. Immunother. Cancer.* 2020; 8, e000299.

75. van Haasteren J, Munis AM, Gill DR, Hyde SC. Genome-wide integration site detection using Cas9 enriched amplification-free long-range sequencing. *Nucleic Acids Res.* 2020; 49, e16–e16.

76. Finlay D, Hor P, Goldenson B, *et al.* Unveiling genomic rearrangements in engineered iPSC lines by optical genome mapping. *Mol. Ther. Methods Clin. Dev.* 2025; 33, 101644.

AFFILIATIONS

Deividas Pažėraitis, Cell Therapy Discovery Biotechnologies, Oncology R&D, AstraZeneca, Cambridge, UK, and Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania

Juozas Kupčinskas, Institute for Digestive Research, and Department of Gastroenterology, Lithuanian University of Health Sciences, Kaunas, Lithuania

AUTHORSHIP & CONFLICT OF INTEREST

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Advocacy at warp speed: delivering the first gene replacement therapy for SLC6A1

INTERVIEW

"We have now de-risked the SLC6A1 transgene and generated human data. That opens the door to many new research opportunities. Someone had to take that leap of faith, and now the data exist"

In September 2025, patient-advocate Amber Freed's son made history to become the first patient to receive a gene replacement therapy for SLC6A1-related neurodevelopmental disorder (SLC6A1-NDD).

Abi Pinchbeck (Editor, *Cell & Gene Therapy Insights*) speaks with Amber Freed (Founder and CEO, SLC6A1 Connect) about patient-led research, the rapid development of an AAV gene replacement therapy for SLC6A1-NDD, and the evolving role of advocacy in rare disease drug development.

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Q For readers unfamiliar with your story, could you briefly explain your patient advocacy journey and the launch of SLC6A1 Connect?

AF My background is in equity research, so I was a professional researcher, but in a financial capacity. Then, my son was diagnosed with SLC6A1 in 2018. At that time, nothing was known about the disease. There was only one paper in existence on the disease at that time. The doctor told us he was one of 34 patients, and we were told to go home and give him the best life possible.

In that moment, I realized that both the blessing and the curse was that there was no current treatment. Nobody was going to help him. That gave us first-mover's advantage. I decided to start a patient organization and lead research at the speed patients need it.

Q Since your **previous interview with *Cell & Gene Therapy Insights*** in 2020, you have reached an extraordinary milestone, with your son becoming the first SLC6A1 patient treated with gene therapy. How do you reflect on that journey, both personally and as an advocate?

AF It is overwhelming to be able to sit here and say that my son received gene replacement therapy. From an advocacy standpoint, it was a truly Herculean effort. We brought together every single patient, built patient registries, funded a natural history study, repurposed a drug, and held an annual scientific symposium, all while advancing drug development.

Drug development is the hardest part when you are dealing with so few patients. All those efforts combined are what got us here. What I like to say about SLC6A1 is that we leapfrogged traditional drug discovery. In six and a half years from launch, we have a gene therapy.

Now, physicians are trying to catch up, and we are backfilling what we do not yet understand about the disease. We are in a position where we can treat the genetic root of a disorder that we do not fully understand clinically. This is a question the entire medical field is facing right now: how much do we need to know about a disease before we can treat it? Patients and parents are ready to move forward, while clinicians are trying to balance caution with urgency as science moves incredibly fast.

Q Could you describe the science behind the AAV-based gene replacement approach for SLC6A1?

AF When I explain this, I always think about other parents who might be reading. I taught myself the basics using *Genetics for Dummies*, *Molecular and Cell Biology for Dummies,* and *Bioinformatics for Dummies*. Mothers can learn very quickly when it is for their children.

The science behind this gene replacement approach is fairly simple and perhaps the most straightforward and eloquent response to the disease, because we are correcting

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"I am championing everyone to make better treatments. We have de-risked this. We are happy to share our data"

the underlying mechanism. Children with SLC6A1 are haploinsufficient. They are missing half of a gene that controls the body's most important inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). Their brains are essentially overexcited. They have epilepsy, movement disorders, autism, and many never speak.

The most direct solution is to replace the missing DNA, in a one-and-done approach. That is exactly what we are doing using AAV9. The virus is delivered into the cerebrospinal fluid and travels into the brain. It targets the cells missing the gene and delivers the functional copy. I often describe it as unclogging a pipe.

The faster we can get this disease on newborn screening panels, the faster we can start getting babies treated. These treatments that patients are driving are going to be the cures of tomorrow.

Q With your son's treatment marking a major milestone, what are your next priorities for the SLC6A1 community?

AF The gene therapy announcement has completely reinvigorated our patient base. It is easy to lose hope in rare disease, because there is always something 'coming soon,' and every day feels like an eternity when a loved one is sick.

We are aiming to initiate a Phase 1/2 clinical trial next year, but that is only the beginning. We hope to license the technology and keep this as a patient-led initiative. Patients need a seat at the table when endpoints are defined, inclusion and exclusion criteria are set, and access is considered.

I am championing everyone to make better treatments. We have de-risked this. We are happy to share our data. I see SLC6A1 as an incubator. Our role is to ensure families have options, understand them, and are empowered to make the right decision for their own situation.

The day after my son's treatment, people asked me if I was going to quit. I said absolutely not. We are just getting started.

Q Which advocacy and community-building strategies were most critical in transforming SLC6A1 into a viable therapeutic program?

AF Advocacy in rare disease is extremely difficult because of that rarity – these disease communities are so small. Parents cannot easily march on Washington with children in wheelchairs. Rare disease families live in a constant gray zone between life and death.

What made the difference for us was fundraising, unity, and focus. We showed our community that if we stayed together, avoided distractions, and focused relentlessly on treatment development, progress was possible. There is also growing strength in rare disease

"Double-blind placebo trials are not feasible, and we need to be more comfortable understanding that attacking the mechanism of disease is the best we are going to get."

communities working together, rather than in isolation. There is a lot of power in the larger rare disease community.

Q How have collaborations with researchers, clinicians, and regulators shaped progress so far?

AF I'm optimistic that the US FDA is beginning to understand the dynamics of rare disease, and that rare disease drug development cannot follow the same rules as more common indications. Double-blind placebo trials are not feasible, and we need to be more comfortable understanding that attacking the mechanism of disease is the best we are going to get.

We need creativity in clinical trial endpoints and much more open communication. We saw what was possible during the COVID-19 pandemic. I hope we can apply that same urgency to rare disease, where these underserved communities have nothing to lose.

Q How can industry and academic groups better integrate patient voices early in program design?

AF I cannot reiterate enough that it is incredibly important to involve patient advocacy groups very early on. I understand the fear of creating false hope, but communication is what creates trust. Advocacy organizations also often have access to a huge number of resources, such as biorepositories, natural history data, and the answers to questions researchers are still asking. We bring together the flywheel of innovation involving researchers, doctors, and patients. A single phone call with an advocate group can accelerate a program dramatically.

Trust takes time. In Texas, we often say it takes a long time to make old friends. If companies engage too late, they risk designing trials that miss what truly matters to patients and may need to redo expensive work.

Q Finally, how can your experience inform progress in other rare diseases and beyond?

AF My journey comes down to grit, determination, and a mother's love. Being told that nothing can be done is never a reason to stop trying. Miracles do not just happen, they are built through thousands of hours of work. Keep going.

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We have now de-risked the SLC6A1 transgene and generated human data. That opens the door to many new research opportunities. Someone had to take that leap of faith, and now the data exist. There is nothing more that pharma could want.

BIOGRAPHY

Amber Freed is the Founder and CEO of SLC6A1 Connect. Beyond her role as a successful leader, Ms Freed is a devoted mother to adorable twins, Miss Riley James and Mr Maxwell Norman. The journey with Maxwell's diagnosis of SLC6A1, a rare neurological disease, became a turning point in Ms Freed's life. With unwavering determination, she left her career in equity research analysis on the day of Maxwell's diagnosis, committing herself to the mission of finding a cure.

Over the course of a few years, Amber has not only navigated the challenges of rare diseases but has also taken on the task of repurposing a drug and succeeding in driving a first in human gene therapy trial. Her dedication and accomplishments have positioned her as a prominent leader within the rare disease community. Join us in this webinar as Ms Freed shares her inspiring story, insights into her journey, and the impactful work being done at SLC6A1 Connect.

Amber Freed, Founder and CEO, SLC6A1 Connect, Frisco, TX, USA

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CELL & GENE THERAPY INSIGHTS

REVIEW

An analysis of advanced therapy medicinal products regulatory landscape in ICH member countries: opportunities and challenges

Srinivasan Kellathur, Joerg HO Garbe, Stuart G Beattie, Martin O'Kane, Anna Litsiou, Kowid Ho, Sarah Adam, Srikanth Muralidharan, and Mustafa Hussain

Advanced therapy medicinal products (ATMPs) represent a class of cutting-edge therapeutic modalities that offer a beacon of hope for treating a range of diseases that were previously incurable. The global demand for ATMPs is increasing, yet the regulatory frameworks and requirements are not harmonized, where several countries are actively working towards establishing specific regulations for this class of innovative therapies. This study analyses some key challenges of ATMP regulations, across International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) regulatory members, and highlights opportunities for regulatory convergence and reliance approaches across multiple jurisdictions to facilitate ATMP development and registration, and ultimately access to patients.

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Advanced therapy medicinal products (ATMPs) have emerged as a promising frontier in medical science, offering potential groundbreaking treatments for a wide range of diseases and conditions with no effective therapeutic options. The regulatory landscape for these innovative therapies is complex and varies significantly across different countries and regions [1–6].

In 2023, the WHO published a considerations document that outlined key principles and concepts for the regulatory oversight of human cells and tissues, and ATMPs [7]. The same year, the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) published a position paper [8] that offered a series of recommendations aimed at promoting convergence and reliance strategies for regulatory submissions concerning ATMPs through clinical trials and the marketing authorization process.

The present study analyses the alignment of recommendations from the IFPMA

position paper with the regulations of ICH member countries. It specifically focuses on the following areas:

- Regulatory landscape for ATMP therapies (regulations, definitions, reliance)
- In-country testing
- Labeling
- ATMPs containing genetically modified organisms (GMOs)

The objective of this analysis is to map which National Regulatory Authority (NRA) have already implemented or plan to implement these recommendations.

Additionally, the study examines the presence of a hospital exemption or similar frameworks for ATMPs within these ICH regulatory members. This is due to the observation that several members have adopted a dual pathway for ATMP development and approval:

- A standard investigational new drug and commercialization route, and
- A pathway for products used within defined parameters in medical practice without the need for a marketing authorization.

METHODOLOGY

The study encompassed ICH regulatory member countries, composed of founding regulatory members (Europe, Japan, USA), standing regulatory members (Canada, Switzerland) and regulatory members (Argentina, Brazil, China, Chinese Taipei, Egypt, Jordan, Republic of Korea, Mexico, Saudi Arabia, Singapore, Turkey, UK) as of December 2024 [9].

A set of survey questions was developed for each area (see Annex 1) based on the recommendations outlined in the IFPMA position paper [8]. Data was collected from agency websites, published literature by agency representatives, and inputs from IFPMA member companies when information was not publicly available (data cut-off date was December 2024). Table 1 summarizes the key themes of the survey questions per area.

SURVEY RESULTS

Regulatory landscape for ATMP therapies (regulations, definitions, and reliance)

Overview

A key challenge in ATMP regulation is the lack of clear, harmonized definitions and product classifications, which are critical to determine the applicability of the corresponding regulations. While several ICH member countries have established specific legal and regulatory frameworks for ATMPs, others are still using the existing medicinal product regulations to manage ATMPs that often do not appropriately fit the unique nature of ATMPs. The 2023 WHO paper on the considerations in developing a regulatory framework for ATMP also emphasizes the need for an understanding of the unique challenges in the development and establishment of a tenable risk-based regulatory framework for the oversight, authorization for marketing and clinical use of ATMPs [7]. Hence, there is a pressing need for fit-for-purpose regulatory frameworks enabling faster access to patients based on clear and harmonized requirements.

Findings

Regulations and definitions

Our analysis revealed that ATMP regulations are rapidly evolving, with several ICH members in the process of developing a regulatory framework.

The definitions and terminology for ATMP are not harmonized across ICH

TABLE 1

Key themes of the survey questions to examine the alignment with IFPMA recommendations and additionally the hospital exemption framework.

Area	Key themes of the survey questions
Regulatory landscape for ATMP therapies (regulations, definitions, reliance)	
Regulations, definitions	Does a country promote harmonization and recognition of product classification?
Reliance	Does a country facilitate reliance, recognition, and collaboration across ATMP lifecycle?
In-country testing	Does a country waive in-country testing?
ATMPs containing GMOs	Does a country harmonize and streamline ATMP-GMO risk assessment?
Labeling	Does a country use an 'universal label' and electronic options?
Hospital Exemption Framework	Has a country a framework for a Hospital Exemption?

ATMP: advanced therapy medicinal product. GMO: genetically modified organism. IFPMA: International Federation of Pharmaceutical Manufacturers and Associations. IFPMA recommendations [8].

members. Canada and Switzerland terms 'advanced therapeutic products' and 'ATMPs', respectively that include a broader range of products than those generally understood categories of ATMPs. Seven ICH members currently lack a regulatory framework, or it is in draft form (Figure 1).

Among those with established ATMP specific regulations, the definitions and terminology are not harmonized as outlined in Table 2 [10–21]. This disparity in definitions and terminology could impact regulatory alignment and consistent application of guidelines across ICH regions.

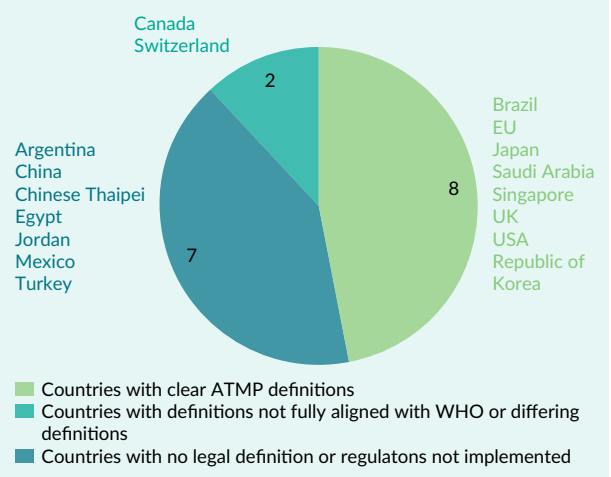
Reliance

The study indicates that there are no formal reliance pathways specific for ATMP Registration/Marketing Authorization across ICH members. Four ICH member regulators have established either reliance pathways or work-sharing procedures for ATMPs. The FDA has initiated a global pilot collaboration on gene therapies, which includes other ICH members. 12 ICH members currently do not have a reliance pathway (Figure 2).

Nevertheless, certain members are applying their existing reliance procedures to ATMPs. As highlighted in the WHO Good Reliance Practices [22], utilizing reliance approaches has the potential to

FIGURE 1

Definitions and terminology in ICH member countries.



The definitions and terminology for ATMP are not harmonized across ICH members. Canada and Switzerland terms 'advanced therapeutic products' and 'ATMPs', respectively that include a broader range of products than those generally understood categories of ATMPs. Seven ICH members currently lack a regulatory framework, or it is in draft form. ATMP: advanced therapy medicinal product. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

TABLE 2

Overview of terminology used in ICH regulatory member countries regulations.

ICH member country	Terminology	Product categories
Brazil, Europe, Saudi Arabia, Switzerland, UK	ATMP	Brazil: advanced cellular therapy products, tissue engineered products and gene therapy products [10] Europe: somatic cell therapy, gene therapy, tissue engineered and combined ATMP [11] Saudi Arabia: same as Europe [12] Switzerland: besides somatic cell therapy products, tissue-engineered products, and gene therapy products, ATMP also includes DNA oligonucleotide, antisense RNA, and vaccines [13] UK: same as Europe [14]
Singapore	CTGTP	Cell therapy, gene therapy, tissue engineering, combined CTGTPs [15]
USA	Regenerative medicine therapy; biological products	Cell therapy, therapeutic tissue engineering product, human cell and tissue product, or any combination product using such therapies or products [16]
Chinese Taipei, Japan	RMP	Chinese Taipei: products containing genes, cells, and their derivatives intended for human use [17] Japan: cell therapy, gene therapy, tissue engineered [18]
The Republic of Korea	ABP	Cell therapy, gene therapy, tissue engineered and combination products [19]
Canada	ATP	ATPs are products that do not fit within Health Canada's existing regulations; they can be either drugs, medical devices, or any combination of both; currently ATP includes fecal microbiota therapy, adaptive machine learning-enabled devices [20,21]
Argentina, China, Egypt, Jordan, Mexico, Turkey	No ATMP specific terminology; existing national frameworks for biologics are applied	N/A

ABP: advanced biopharmaceutical products. ATMP: advanced therapy medicinal products. ATP: advanced therapeutic products. CTGTP: cell, tissue, and gene therapy products. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. RMP: regenerative medicine products.

enhance patient access to essential medicines. For instance, in Singapore, a CTGTP can qualify for an abridged evaluation if it has already received approval from at least one of the HSA's comparable overseas regulators [23]. Similarly, the UK Medicines and Health Products Regulatory Agency (MHRA) implemented an International Recognition Procedure, which allows marketing authorization application for products, including ATMPs, already authorized by one of MHRA's designated reference regulators [24].

There are work-sharing and collaborative mechanisms specifically for ATMP that are being developed, such as the ACCESS consortium and CoGenT (Collaboration on Gene Therapies) Global Pilot. In December 2023, the ACCESS coalition expanded its scope to include ATMPs [25]. This aims to enhance the harmonization of regulatory approaches for ATMPs across these agencies. Furthermore, in 2024, the FDA launched the CoGenT Global Pilot that is exploring the potential for concurrent, collaborative reviews of new gene therapy applications with other international regulatory bodies (the EMA, FDA, PMDA, Health Canada and Swissmedic).

Several countries, including Singapore and Japan, have established conditional approval pathways specifically for ATMPs [15,18]. Furthermore, programs designed to expedite product approvals, such as the Regenerative Medicine Advanced therapy (RMAT) designation in the USA, the SAKIGAKE designation in Japan, and the PRIME (Priority Medicines) scheme in Europe, offer significant support for manufacturers developing products intended

for serious conditions or unmet medical needs.

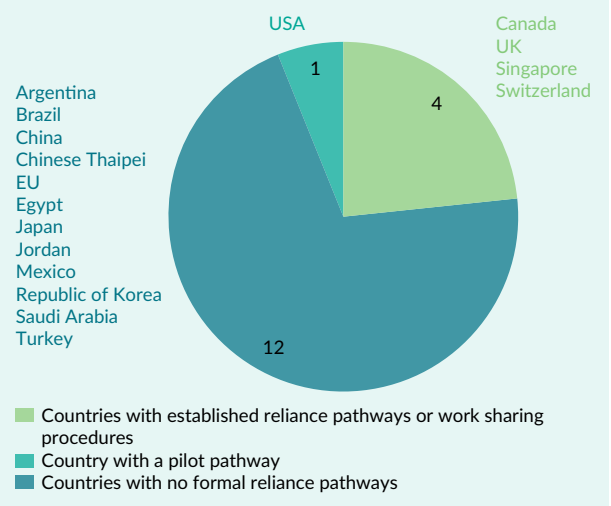
Recommendations

- Establish appropriate ATMP framework with clear definitions/classification: the regulatory oversight of ATMPs necessitates a robust framework. This is crucial for addressing the unique challenges associated with ATMPs, particularly in clinical trials, manufacturing, and pharmacovigilance. The correct classification of ATMPs plays a vital role in several key aspects. It is essential for safeguarding patient safety by applying the appropriate regulatory levers, it streamlines the regulatory processes that these innovative therapies undergo, and it provides clear direction for their development pathways, among others. It also facilitates access to the various services and incentives offered by health authorities. These schemes enable developers to initiate a dialogue with regulators that helps them receive valuable guidance on the regulatory requirements and clinical development strategies;

- Facilitate reliance, recognition, and collaboration across ATMP lifecycle: to improve access to high-quality, safe, and effective ATMPs, greater collaboration among regulatory bodies at both regional and global levels is important across the entire product lifecycle, from early clinical development through marketing authorization and post-approval changes. This includes working through regulatory networks to share knowledge, exchange experiences, and utilize resources more efficiently. The alignment of regulatory requirements across different regions would enhance efficiencies and foster opportunities for regulatory reliance [26]. Such reliance is particularly vital for expanding access to ATMPs, as many countries' regulatory authorities currently have limited or no precedent in authorizing these innovative medicinal products.

FIGURE 2

Reliance and work-sharing procedures for ATMPs.



Four ICH member regulators have established either reliance pathways or work-sharing procedures for ATMPs. The US FDA has initiated a global pilot, collaboration on gene therapies, that includes other ICH members. Twelve ICH members currently do not have a reliance pathway. ATMP: advanced therapy medicinal product. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

In-country testing

Overview

Secondary quality control of traditional pharmaceutical products is required in several markets [27] and is associated with multiple implications [28–30]. In-country quality control is performed for:

- Registration testing, including lifecycle management (new registrations, license renewals, line extensions, post-approval changes) [31], as well as

- Import testing (including lot release testing of vaccines and blood products) [32]

With all their inherent below-outlined challenges, ATMPs require streamlined, effective and predictable registration and importation processes ensuring registration compliance as well as authenticity, quality, and suitability.

In-country testing for ATMPs presents unique challenges due to their nature [33]:

- Small batch sizes, often for (very) small patient populations or a single patient
- High consumption of a batch for testing, reducing the amount available for patient treatment
- Delayed patient access due to lead times for method implementation and duplicative testing
- Supply chain issues related to country-specific testing requirements
- Chain of custody: Import testing would cause additional activities (e.g., product transportation to control laboratory, longer hold time), introducing risks to cold chain interruptions and patient supply.

Findings

Our analysis of registration testing revealed that two out of 17 countries do not allow waivers, causing significant implications for patient access and batch consumption. Five countries allow waivers under conditions or partial waivers; 10 countries have no testing requirements or apply systemic waivers (Figure 3).

For import testing one country does not allow waivers, while 10 countries allow waivers under conditions or partial waivers; six countries have no testing requirements or apply systemic waivers (Figure 4). Even if conditional/partial waivers are possible, the discretionary nature of these waivers leads to uncertainty whether testing is required and therefore must be implemented in a country until the end of the registration process.

Recommendations

- Apply waiver schemes for in-country testing based on reliance on product approvals and inspections from mature NRAs (mature NRAs refers to Stringent Regulatory Authorities, SRAs. A list of SRAs has been published by the WHO [34]. Once the WHO listed authority (WLA) system is fully implemented the term WLA will replace the term SRA;
- Rely on Certificates of Analysis (CoAs) issued by manufacturers in facilities inspected by mature NRAs. This ensures that the manufacturer:
  - Provides evidence that the product manufacturing, testing, and storage/distribution systems are well controlled, validated and comply with current GMP/GDP;
  - Has implemented a proper Quality Management System (QMS) to ensure compliance;
  - Is under regular control of independent auditing and globally recognized inspectorates (e.g., PIC/S members or other competent NRAs).

ATMPs containing genetically modified organisms (GMOs)

Overview

In many regions, GMO regulations (requirements and procedures) were primarily established for agricultural applications and have also been applied to GMO ATMPs, that is, ATMPs that consist of, or contain, GMOs. GMO risk assessment requirements that are appropriate for deliberate release of GMO plants which may be able to propagate and be released across wide areas,

do not appropriately reflect the negligible risk to the environment when ATMPs are developed through clinical trials in controlled environments. Negligible risks to the environment are determined through an Environmental Risk Assessments (ERA). An ERA requires a scientific understanding of the survival characteristics and replication competency of the GMO or vector used to modify human cells, together with non-clinical and (if available) clinical knowledge regarding pharmacokinetics and shedding, post-administration [35,36].

The regulation of ATMPs containing GMOs is highly fragmented across regions, particularly at the clinical trial stage. Procedures, GMO risk assessment data requirements, review, and approval timelines vary significantly, resulting in a high regulatory burden and potential delays in starting clinical trials, as reported for the EU [37].

Findings

There are significant differences in ERA requirements across (ICH) regulatory members. The FDA allows a claim of 'categorical exclusion' for clinical studies with gene therapies [38], exempting them from environmental assessment requirements for Investigational New Drugs. In contrast, there are lengthy procedures and data requirements in the EU, Brazil, Canada, and Japan. However, in Canada a single assessment can be performed on a *per product* basis, rather than a *per trial* basis.

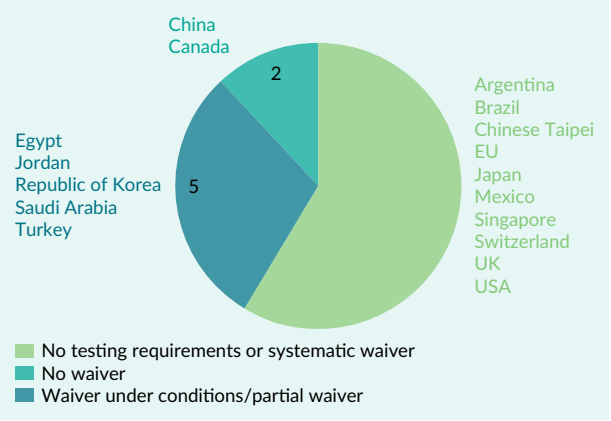
Adjacent to a Clinical Trial Application (CTA), there are specific GMO application review timeframes defined by national GMO competent authorities in some ICH regulatory member regions (Table 3) [39,40]. In some regions, depending on the ATMP, review and approval for use of investigational medicinal products that contain or consist of GMOs may require liaison or consultation with additional authorities.

There are potential delays to clinical trial initiation due to GMO-related requirements. There has not been a survey of developer's experiences across all ICH regions, yet there have been three industry surveys across the EU:

- In 2021, an industry survey found that GMO approval timelines were highly variable, often within the same EU Member State. Further, 32% of GMO approvals by GMO competent

FIGURE 3

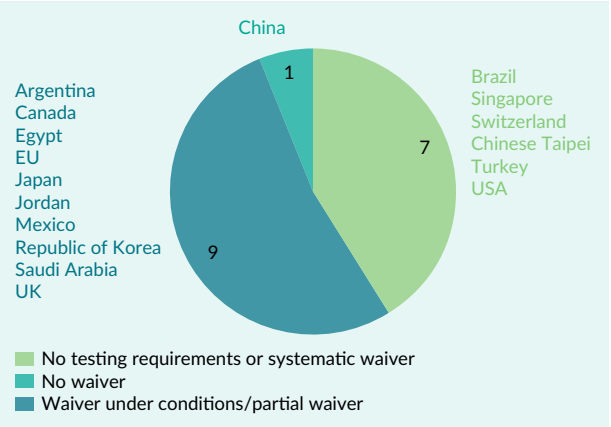
In-country quality control performed for registration testing in ICH member countries.



ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

FIGURE 4

In-country quality control performed for import testing in ICH member countries.



ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

TABLE 3

GMO application review timeframes in ICH regulatory member countries.

Country	Timeframe	Comments
Brazil	8–10 months	5 months if urgent review requested
Canada	120 days	Intended efforts to align assessment period to match CTA 30 days review period
European Union	15–165 days	Variable depending on member state; review within 30 days observed in some countries (e.g., France, Belgium, and Portugal)
Japan	≥3 months–6 months	If human cells are genetically modified cells using recombinant viruses of the family Retroviridae (gammaretroviruses and lentiviruses, designed so that replication-competent recombinant viruses are not easily generated) and if it can be demonstrated that the final product does not contain residual active vector, then a GMO/LMO (living modified organism) exemption can be claimed [38,39]
UK	45–100 days	For low hazard, class 1 (such as rAAV): final clearance within 45 days; however, if the clinical site has prior contained use notification, no further notification is required Class 2: up to 55 days without prior notification; up to 10 days if prior site notification Class 3-4: up to 100 days without prior site notification; up to 55 days if prior site notification
China, Chinese Taipei, Egypt, Mexico, Republic of Korea, Singapore*, and Turkey	N/A	No regulatory framework specific to ATMPs containing GMOs

*Genetic Modification Advisory Committee recommendation is required adjacent to a CTA for a CTGTP. ATMP: advanced therapy medicinal products. CTA: Clinical Trial Application. GMO: genetically modified organism. LMO: living modified organism. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

authorities were granted after approval of the CTA (under the old EU Clinical Trials Directive) [37];

- A 2023 survey of European Federation of Pharmaceutical Industries and Associations (EFPIA) member companies showed that there are highly variable timeframes (15–165 days) with differences in adaptation to timelines dictated by the EU Clinical Trials Regulation (that replaced the Clinical Trials Directive). Industry-specific survey responses revealed how specific member states (Czechia and Poland) are de-selected by sponsors as regions to host a clinical trial, since GMO authorization is required prior to acceptance of CTA. Delays have been reported for EU Member States, where 60% survey respondents reported that their GMO application delayed initiation to a clinical trial [41]. Despite the adoption of harmonized (common application) forms for GMO applications, the benefits of a single CTA submission under the CTR are diminished since CTA coordination under the CTR does not facilitate multi-Member State GMO applications;
- In an unpublished EFPIA follow-up survey from 2025, 90% respondents report that there is no alignment across review timelines for a GMO application and CTA. Misalignment caused delays to the initiation of clinical trials in 44% cases, with delays of ≥100 days and 51 days reported for Hungary and Belgium, respectively;
- Through Revision to the EU General Pharmaceutical Legislation, the European Commission (EC) have proposed that ERAs are to be assessed

centrally by the European Medicines Agency's Committee for Medicinal Products (CHMP) for Human Use. This removes the disparate interpretations of GMO Directives by EU Member State GMO Competent Authorities, with a single CHMP review timeframe [42]. Separately, the EC European Biotech Act includes a proposal to exempt investigational GMO ATMPs from requiring an ERA if they pose no or negligible risks to the environment [43].

Other than Brazil which recognizes EU GMO application packages, there is a lack of reliance or recognition procedures for GMO Investigational Medicinal Product authorization prior to, or in parallel to a CTA.

Another issue faced by developers is that there is not a single definition of a GMO or LMO, applied across all regions. For example, this can lead to the lack of clarity on the need to submit GMO applications and ERAs for some modalities, such as Viral Replicon Particles.

Recommendations

- Develop a global or universal common application forms for genetically modified cells or viral vectors: this could minimize duplication of efforts with associated different data requirements. Existing ERA could serve as basis for global considerations [44];
- Foster reliance or developing a legal framework for GMO ATMPs: It is proposed that recognition and reliance pathways could be explored by international regulators based and developed upon the Cartagena Protocol on Biosafety [45] and its supplementary protocol on liability and redress. Adherence to the Cartagena protocol would need to be established when seeking reliance procedures.

Labeling

Overview

ATMP labeling is governed by the packaging and labeling for medicinal products across jurisdictions presenting unique challenges that differentiate it from conventional pharmaceutical labeling. ATMP products require specialized labeling that addresses their biological nature, patient-specific applications, and complex manufacturing processes [43]. Some key challenges identified regarding ATMP labeling are as follows: the need to accommodate ultra-low temperature storage requirements, and navigating country-specific regulatory compliance across different jurisdictions; labeling system must also ensure traceability through unique identifiers while maintaining patient privacy, labels must convey comprehensive safety information, handling instructions, and product characteristics despite space limitations; and the system also needs to support real-time manufacturing environments and distributed clinical settings [46].

Findings

The analysis focused on the availability of guidelines for ATMP labeling, international standards for track and trace, such as ISBT128 [47] use of digital technology for e-labeling, and language requirements. (ISBT 128 is an international standard for the identification, coding, labeling, and information transfer of human blood, cell, tissue, and organ products. It provides a globally unique donation numbering system, standardized product codes, and data structures for barcoding and electronic data interchange, ensuring accuracy, safety, and efficiency across international borders and different healthcare systems. The system uses a 13-character unique donation identifier and supports machine-readable bar codes such as Code 128 and Data Matrix symbols. It is managed by ICCBBA, a nonprofit organization, and is widely adopted worldwide for improving traceability and

patient safety in transplantation and transfusion).

On the availability of guidelines for ATMP labeling, the analysis indicated that 41% of ICH members (7/17) have published specific guidelines on ATMP labeling while the remaining ICH members refer to the packaging and labeling requirements for medicinal products. This presents a clear opportunity to develop a more harmonized regulatory framework for ATMP labeling (Figure 5).

Packaging and labeling for ATMPs are notably impacted by product characteristics, including the requirement for ultra-low temperature storage for certain ATMPs (e.g., ≤−150 °C), which presents durability challenges for conventional labels, particularly those affixed to primary containers such as vials or cryo-bags prior to freezing. Examining whether ICH members have specific ATMP labeling statements on product information and packaging, the survey found that 41% (7/17) of members have distinct packaging requirements for ATMPs, while two member countries have such requirements only in specific cases; the majority of ICH member counties follow conventional pharmaceutical labeling requirements. The results indicate that the nature of ATMP necessitates specific information on the labels (Figure 6).

ATMPs, in particular cell-based products, are often personalized medicine, and tracking the information end to end from donation, to manufacturing, to product release, and administration at the clinical site is key for accuracy, safety, and efficiency. Information Standard for Blood and Transplant 128 (ISBT 128) is an international standard for the identification, coding, labeling, and tracking of medicinal products of human origin [48]. The adoption of ISBT 128 standard remains limited with only 23% (4/17) of the regulators implementing this standard to some extent (Figure 7).

Tracking, traceability, and having a common language on the label are also key trends for harmonization and reaching all stakeholders. We analyzed the data on specific guidance for track and trace, the use of mobile technologies such as QR codes and 2D matrices, and the acceptability of the English language on primary packaging. Regarding patient traceability, 35% (6/17) of ICH members have specific track and trace requirements, with the Republic of Korea also having requirements in certain cases (Figure 8). Additionally, 47% (8/17) of members permit or encourage the integration of mobile technology, such as QR codes or 2D matrix codes, for labeling, which facilitates electronic product information and enhances traceability systems (Figure 9).

Furthermore, the acceptability of English on primary labeling across ICH members demonstrates flexibility and diversity. This is particularly relevant due to the challenges associated with ATMP labeling, especially the limited space on primary packaging. Our data shows that 41% (7/17) of members allow the use of English on primary packaging, while another 41% (7/17) permit flexibility after product-specific discussions with NRAs (Figure 10).

Recommendations

- Broaden implementation of international standards: regulatory agencies should consider developing harmonized labeling standards for ATMPs, or considering the adoption of ISBT128, to facilitate traceability, improve patient safety, and support global distribution of ATMPs;
- Develop tailored and adaptive regulatory frameworks: legislation and guidance should incorporate specific provisions for the unique requirements of ATMPs, including considerations for ultra-low temperature storage and the durability of label materials under such conditions;

- Enable flexible labeling approaches: regulators should allow for adaptable language and content on primary and secondary packaging to address multilingual markets and reduce barriers to access, while ensuring the clarity and accuracy of product information;

- Leverage digital technologies: integration of mobile technologies (e.g., QR codes or 2D matrix codes) should be encouraged to provide real-time access to electronic product information, enhance track-and-trace systems, and support pharmacovigilance.

Hospital exemption framework

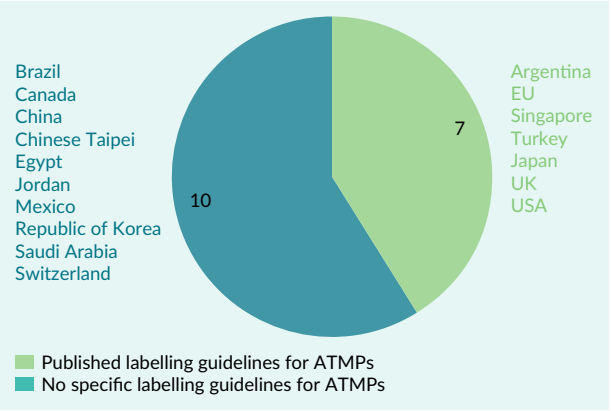
Overview

There is a lack of internationally standardized regulatory mechanisms to support the provision of unlicensed cell, gene, or tissue therapies to patients with unmet medical needs. In the EU, there is a regulatory mechanism under EU law that allows certain ATMPs to be used without a centralized marketing authorization under specific conditions. Article 28(2) of the EU ATMP Regulation No 1394/2007, as implemented in Article 3(7) of Directive 2001/83, introduces the Hospital Exemption scheme, which empowers Member States to permit the provision of an advanced therapy medicinal product, without marketing authorization, under certain conditions. Specifically, these products must be prepared on a non-routine basis; prepared in accordance with specific quality standards; used within a single Member State; used in a hospital setting, under the exclusive professional responsibility of a medical practitioner; and prepared to comply with a medical prescription for a custom-made product for an individual patient.

EU Member States must authorize the manufacturing of these products and ensure manufacturing quality standards are met. They must also ensure traceability and pharmacovigilance requirements are met.

FIGURE 5

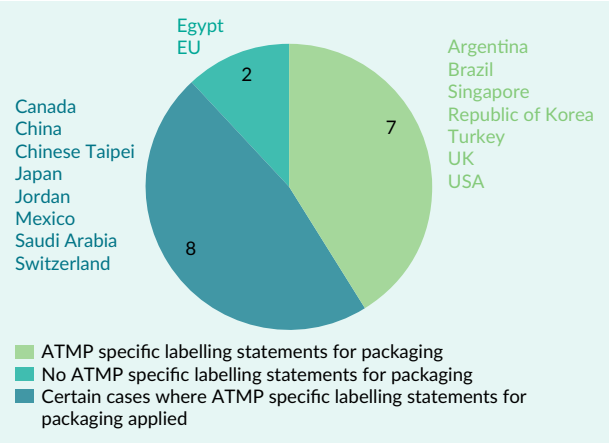
Published labeling guidelines for ATMPs.



Seven ICH member countries have issued ATMP-specific labeling guidelines. The remaining ICH members currently use the existing labeling guidelines provided for prescription medicines. ATMP: advanced therapy medicinal product. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

FIGURE 6

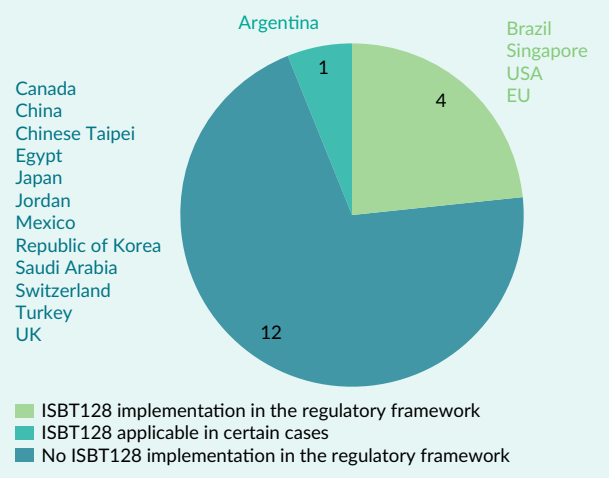
Distinction between countries that mandate specific statements on ATMP packaging.



Seven countries require ATMP-specific labeling statements on product packaging as part of their regulatory compliance. Egypt and the EU only require such statements in certain special cases. Eight jurisdictions do not require any ATMP-specific labeling statements for packaging. ATMP: advanced therapy medicinal product.

FIGURE 7

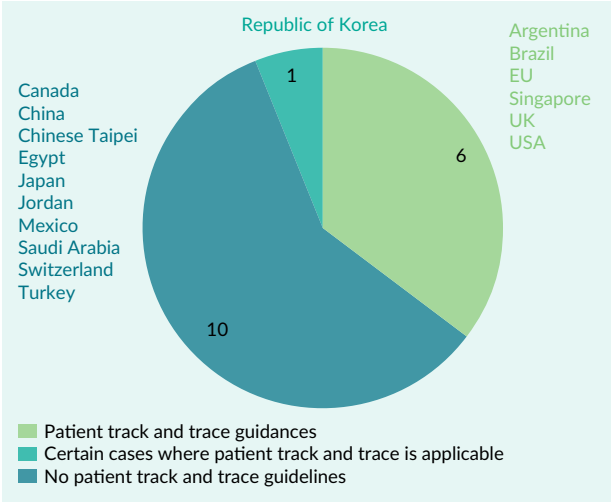
Global implementation of ISBT128 for ATMPs within national regulatory frameworks.



Currently, Brazil, Singapore, and the USA have fully implemented ISBT128 within their national regulations for ATMPs. Argentina and the EU have partial or conditional ISBT128 implementation. Twelve countries do not require or reference ISBT128 in their ATMP regulations. ATMP: advanced therapy medicinal product. ISBT 128: International Society of Blood Transfusion standard 128

FIGURE 8

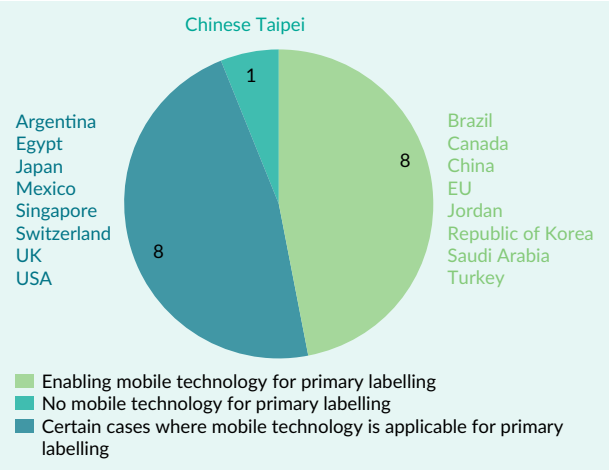
Patient track and trace mechanisms for ATMPs in ICH member countries.



Six members mandate patient track and trace in their regulations. The Republic of Korea requires only in specific cases. Ten countries currently do not have specific regulatory guidance on patient track and trace for ATMPs. ATMP: advanced therapy medicinal product. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

FIGURE 9

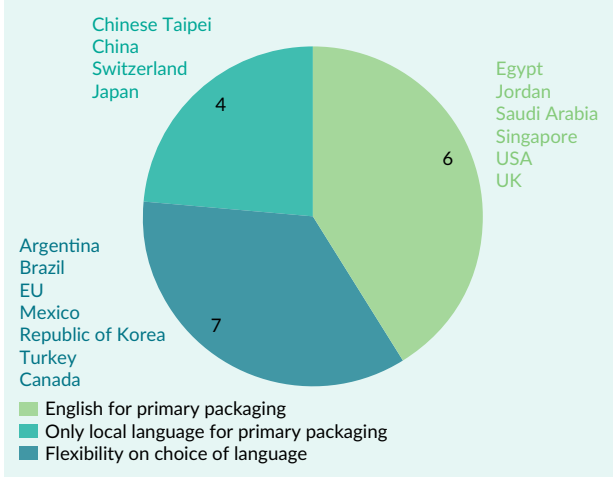
Diversity in regulatory approaches to digital innovation in labeling practices.



Eight ICH members support the use of mobile technology on the primary package of ATMPs, while eight members allow only in specific cases. Chinese Taipei does not support the use of mobile technology on the ATMP primary package. ATMP: advanced therapy medicinal product. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

FIGURE 10

ATMP language requirements for primary packaging in ICH member countries.



Six ICH member countries permit the use of English as the language for primary packaging. Seven members offer flexibility, allowing either English or the local language depending on specific circumstances or context. Four members strictly mandate the use of the local language for primary packaging. ATMP: advanced therapy medicinal product. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

Findings

The study conducted a landscape analysis of international equivalents to the EU Hospital Exemption (HE) scheme, which allows for the provision of unlicensed ATMPs under certain conditions. For countries with provisions, both Health Authority approval and good manufacturing practice (or equivalent) compliance are the most common requirements. In Japan, as well as Health Authority approval, the manufacturing standards share the same principles as good gene, cellular, and tissue-based products manufacturing practice (GCTP) or good manufacturing practice (GMP).

In the UK the product to be used under Hospital Exemption is not directly approved by the Health Authority but manufacture must be conducted to GMP.

In the USA, there is no provision for ATMP administration under the hospital exemption pathway. However, access to unlicensed ATMPs is via an Investigation New Drug (IND) pathway while in Canada an equivalent to the EU HE scheme does not exist, but an alternative pathway allows access via the Special Access Program for Drugs.

In Switzerland, while there is currently no formal implementation of an equivalent to HE, manufacture of unlicensed products in a hospital pharmacy under a manufacturing license is allowed, and relevant legislation is under review which may introduce similar provisions as for EU HE.

The Republic of Korea endorsed a new law in 2020 (anticipated to be applicable in 2025) allowing patients with severe, rare, or incurable conditions to receive cell and gene therapies that do not have market approval following approval of safety and treatment plans.

Chinese Taipei and Saudi Arabia do not have specific provisions. Overall, the processes and requirements differ from country to country, and it should also be noted that although the EU was captured as a bloc, it has been recognized that the hospital exemption scheme has been interpreted and implemented inconsistently across Member States [49].

Recommendations

- Foster collaboration, convergence, and ultimately consistency among regulators in the area of hospital exemption or equivalent schemes to facilitate access to unlicensed ATMPs: this would result in individual patients being able to access treatment where there is an urgent unmet clinical need, and no where there is no available product on the market in their territory or no opportunity to take part in a suitable clinical trial;

- Encourage establishment of an internationally aligned and robust regulatory pathway to facilitate commercial and non-commercial product development via appropriately robust and regulated pathways, according to established licensing routes for evidence generation, avoiding dual pathways with different quality, safety, and efficacy standards.

CONCLUSION & FUTURE OPPORTUNITIES

While many ICH member countries have adopted scientifically sound, risk-based approaches to ATMP regulation, several members continue to apply standard pharmaceutical product regulations to these product categories. The diverse nature of ATMPs necessitates regulatory frameworks that are flexible and adaptable to accommodate their unique characteristics. These principles also extend to non-ICH member countries and may lay the groundwork for reliance models, especially in low- and middle- income countries (LMICs).

To foster unilateral reliance and recognition approaches, avoid duplication of

efforts, and encourage efficient resource utilization, there is a need for:

- Convergence of regulatory approaches (e.g., definition of products, classification, regulatory requirements) as well as embrace reliance and work-sharing procedures, while maintaining sovereignty of regulatory decision making;
- Waiving in-country testing for ATMPs: relying instead on CoAs issued by manufacturers operating in facilities approved and inspected by mature NRAs;
- Standardized approaches to ERA of GMO-ATMPs: developing standardized approaches for the application form and ERA are crucial to streamline processes and reduce regulatory burden;
- Convergence on labeling requirements: continued collaboration among regulatory authorities is needed to harmonize labeling requirements, reduce redundancies, and optimize the regulatory pathway for ATMPs, ultimately facilitating timely patient access to advanced therapies;
- The implementation of consistent and internationally aligned regulations regarding hospital exemptions and equivalent schemes is essential. Ensuring clarity across these frameworks is necessary to guarantee the quality, safety, and efficacy of products.

Continued collaboration among regulatory authorities and the WHO is paramount to developing fit-for-purpose ATMP regulations and requirements. As the field continues to rapidly evolve, ongoing efforts to harmonize and streamline regulatory processes will be crucial to ensure that these innovative treatments can reach patients in need across the globe.

REFERENCES

1. Blaese RM, Culver KW, Miller AD, *et al.* T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* 1995; 270, 475–480.
2. American Society of Gene and Cell Therapy. Gene, cell, and RNA therapy landscape report. 2025. https://www.asgct.org/global/documents/asgct-citeline-q1-2025-report.aspx (accessed Sep 22, 2025).
3. Frederico CC. Advanced therapy medicinal products development – from guidelines to medicines in the market. *Biotechnol. Adv.* 2025; 83, 108612.
4. Wang W, Smith A. Enhancing the clinical translation of stem cell models by focusing on standardization and international regulatory cooperation. *World J. Stem Cells* 2025; 17, 102788.
5. Horgan D, Arena-Peréz MA. Propelling healthcare with advanced therapy medicinal products: a policy discussion. *Biomed. Hub* 2020; 5, 130–132.
6. Shi J, Chen C. The evolving regulatory system of advanced therapy medicinal products in China: a documentary analysis using the World Health Organization global benchmarking tool standards. *Cytotherapy* 2024; 26, 954–966.
7. World Health Organization. Considerations in developing a regulatory framework for human cells and tissues and for advanced therapy medicinal products. 2023. https://cdn.who.int/media/docs/default-source/biologicals/annex-3---hcts-atmps-regulatory-considerations---clean-for-posting---12-may-2023.pdf?sfvrsn=cc1f9a8a_1&download=true (accessed Sep 22, 2025).

8. International Federation of Pharmaceutical Manufacturers and Associations. Points to consider to promote convergence and reliance approaches for advanced therapy medicinal products. 2023 https://www.ifpma.org/wp-content/uploads/2023/02/2022-position-paper-ATMP-convergence-and-reliance-ifpma-en.pdf (accessed Sep 22, 2025).
9. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Current members and observers. 2024. https://www.ich.org/page/members-observers (accessed Aug 4, 2024).
10. Junior JBS, Pereira RM. Advanced therapy products in Brazil: regulatory aspects. *Adv. Exp. Med. Biol.* 2023; 1430, 117–133.
11. Schuessler-Lenz M, Harder C. Marketing regulatory oversight of advanced therapy medicinal products in Europe. *Adv. Exp. Med. Biol.* 2023; 1430, 1–21.
12. Saudi Food and Drug Authority. Guideline on classification of advanced therapy medicinal products. 2023. https://www.sfda.gov.sa/en/regulations/89333 (accessed Sep 22, 2025).
13. Bukovac PK, Huwyler M. The regulation of cell therapy and gene therapy products in Switzerland. *Adv. Exp. Med. Biol.* 2023; 1430, 41–58.
14. Medicines and Healthcare products Regulatory Agency. Advanced therapy medicinal products: regulation and licensing in UK. 2025. https://www.gov.uk/guidance/advanced-therapy-medicinal-products-regulation-and-licensing#overview (accessed Sep 22, 2025).
15. Ong LL. Regulatory oversight of cell, tissue, and gene therapy products in Singapore. *Adv. Exp. Med. Biol.* 2023; 1430, 197–210.
16. Sanduja S, Lee-Morrison L. United States Food and Drug Administration regulation of human cells, tissues, and gene therapies. *Adv. Exp. Med. Biol.* 2023; 1430, 71–89.
17. Chinese Taipei. Regenerative medicine act. 2024. https://law.moj.gov.tw/ENG/LawClass/LawAll.aspx?pcode=L0020238 (accessed Sep 22, 2025).
18. Maruyama Y, Nishimura S. Regulatory aspects of cell and gene therapy products: the Japanese perspective. *Adv. Exp. Med. Biol.* 2023; 1430, 155–179.
19. Ministry of Food and Drug Safety. Act on the safety of and support for advanced regenerative medicine and advanced biological products. 2025. https://elaw.klri.re.kr/eng_mobile/viewer.do?hseq=56486&type=part&key=37 (accessed Sep 22, 2025).
20. Wang J, Gagliardi E. Canadian regulatory framework and regulatory requirements for cell and gene therapy products. *Adv. Exp. Med. Biol.* 2023; 1430, 91–116.
21. Health Canada. Advanced therapeutic products. 2025. https://www.canada.ca/en/health-canada/services/drug-health-product-review-approval/advanced-therapeutic-products.html (accessed Sep 22, 2025).
22. World Health Organization. WHO TRS 1033 – annex 10: good reliance practices in the regulation of medical products: high level principles and considerations. 2021. https://www.who.int/publications/m/item/annex-10-trs-1033 (accessed Sep 22, 2025).
23. Health Sciences Authority. Class 2 CTGTP registration. 2024. https://www.hsa.gov.sg/ctgtp/registration (accessed Sep 22, 2025).
24. Medicines and Healthcare products Regulatory Agency. International recognition procedure. 2025. https://www.gov.uk/government/publications/international-recognition-procedure/international-recognition-procedure (accessed Sep 22, 2025).
25. ACCESS Consortium. ATMP working group. 2025. https://accessconsortium.info/homepage (accessed Sep 22, 2025).
26. Colmagne Poulard I, Ausborn S, Harvey Allchurch M, *et al.* Reliance into action: understanding EMA documents to streamline reliance for marketing authorization applications. *Ther. Innov. Regul. Sci.* 2025; 59, 1032–1041.
27. Garbe JHO, Ennis K, Furer G, Jacobs M, Roenninger S. Import testing of pharmaceutical products has limited safety benefits and can add risk to patients. *Pharm. Tech.* 2015; 09-SP, s18–s26.

28. Roenninger SK, Garbe JHO. Import testing turned into an unnecessary limitation of patient access to medicines as risks are managed effectively. *Pharm. Policy Law* 2016; 18, 141–156.

29. Garbe JHO, Jacobs M, Roenninger S. Import testing: an outdated practice? Opportunities for improved access to safe and efficient medicines. *Ther. Innov. Regul. Sci.* 2017; 51, 620–624.

30. Garbe JHO, Conti T, McGoldrick M, *et al.* Reliance-based waivers become the predominant trend in in-country testing of pharmaceutical products. *Pharm. Tech.* 2024; 48, 18–28.

31. International Federation of Pharmaceutical Manufacturers and Associations. Best practices for in-country testing and sample management. 2020. https://www.ifpma.org/publications/position-paper-best-practices-for-in-country-testing-and-sample-management (accessed Sep 22, 2025).

32. International Federation of Pharmaceutical Manufacturers and Associations. Appropriate control strategies eliminate the need for redundant testing of pharmaceutical products. 2016. https://www.ifpma.org/publications/import-testing-position-papers (accessed Sep 22, 2025).

33. International Federation of Pharmaceutical Manufacturers and Associations. In-country testing of advanced therapy medicinal products. 2022. https://www.ifpma.org/publications/position-paper-in-country-testing-of-advanced-therapy-medicinal-products (accessed Sep 22, 2025).

34. ICH: WHO-Listed Authority (WLA). www.who.int/initiatives/who-listed-authority-reg-authorities.

35. European Medicines Agency. Guideline on environmental risk assessments for medicinal products consisting of, or containing, genetically modified organisms (GMOs). 2006. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-environmental-risk-assessments-medicinal-products-consisting-or-containing-genetically-modified-organisms-gmos_en.pdf (accessed Sep 22, 2025).

36. Baldo A, van den Akker E, Bergmans HE, Lim F, Pauwels K. General considerations on the biosafety of virus-derived vectors used in gene therapy and vaccination. *Curr. Gene Ther.* 2013; 13, 385–394.

37. Beattie S. Call for more effective EU regulation of clinical trials with advanced therapy medicinal products consisting of or containing genetically modified organisms. *Hum. Gene Ther.* 2021; 32, 997–1003.

38. US Food and Drug Administration. Determining the need for and content of environmental assessments for gene therapies, vectored vaccines, and related recombinant viral or microbial products. 2015. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/determining-need-and-content-environmental-assessments-gene-therapies-vectored-vaccines-and-related (accessed Sep 22, 2025).

39. Mizoguchi H, Fleischmann T, Komuro M, *et al.* Comparative analysis of GMO regulatory requirements for AAV vectors in the EU and Japan focusing on the shedding data and containment measures. *Expert Opin. Biol. Ther.* 2024; 24, 529–542.

40. Bachtarzi J, Fudge T. The genetically modified organism medicinal framework in Europe, United States and Japan: underlying scientific principles and considerations toward the development of gene therapy and genetically modified cell-based products. *Hum. Gene Ther. Clin. Dev.* 2019; 30, 114–128.

41. Beattie SG, Lambot N, Awigena-Cook J, *et al.* Clinical trial applications for investigational medicinal products that contain or consist of genetically modified organisms: industry experience under the European Union clinical trial regulation (536/2014). *Cell Gene Ther. Insights* 2024; 10, 375–395.

42. European Commission. Regulation of the European Parliament and of the Council laying down Union procedures for the authorisation and supervision of medicinal products for human use and establishing rules governing the European Medicines Agency, amending Regulation (EC) No 726/2004, Regulation (EC) No 141/2000, and Regulation (EC) No 1901/2006. 2023.

43. European Commission. Regulation of the European Parliament and of the Council on establishing a framework of measures for strengthening Union's biotechnology and biomanufacturing sectors particularly in the area of health and amending Regulations (EC) No 178/2002, (EC) No 1394/2007, (EU) No 536/2014, (EU) 2019/6, (EU) 2024/795 and (EU) 2024/1938 (European Biotech Act). 2025.

44. European Medicines Agency Committee for Medicinal Products for Human Use. Guideline on scientific requirements for gene therapy medicinal products. 2008. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-scientific-requirements-environmental-risk-assessment-gene-therapy-medicinal-products_en.pdf (accessed Sep 22, 2025).

45. Convention on Biological Diversity. Parties to the Cartagena protocol and its supplementary protocol on liability and redress. 2018 https://bch.cbd.int/protocol/parties (accessed Sep 22, 2025).

46. European Group for Blood and Marrow Transplantation Gene and Cell Therapy Committee. Packaging and labelling of ATMPs. 2024. https://www.eurogct.org/research-pathways/manufacturing/packaging-and-labelling-atmps (accessed Sep 22, 2025).

47. International Society of Blood Transfusion. What is ISBT128? 2025. https://www.isbt128.org/isbt-128-basics (accessed Sep 22, 2025).

48. Hagen H, Suchet C. Small labels, big challenges: solutions for advanced therapy labelling. *Cell Gene Ther. Insights* 2020; 6, 1183–1195.

49. European Federation of Pharmaceutical Industries and Associations. Joint position on hospital exemption scheme. 2023. https://www.efpia.eu/media/1wwnhpem/arm-efpia-europabio-eucope-isct-joint-paper-on-hospital-exemption-scheme-july-2023.pdf (accessed Sep 22, 2025).

AFFILIATIONS

Srinivasan N Kellathur, Pharma Technical Regulatory Policy APAC, Roche Singapore Technical Operations, Singapore

Joerg HO Garbe, Global Quality and Compliance, Pharma Technical Operations, F Hoffmann-La-Roche Ltd, Basel, Switzerland

Stuart G Beattie, Regulatory Affairs Chemistry, Manufacturing and Controls, Biogen, UK

Martin O'Kane, RA EU Policy and Liaison, Novartis Pharmaceuticals UK Ltd, UK

Anna Litsiou, International Regulatory Policy and Intelligence Lead, AstraZeneca UK Limited, UK

Kowid Ho, International Pharma Technical Regulatory Policy, F Hoffmann-La-Roche Ltd, Basel, Switzerland

Sarah Adam, International Federation for Pharmaceutical Manufacturers (IFPMA), Geneva, Switzerland

Srikanth Muralidharan, Regulatory Information Management Systems, AstraZeneca India Private Limited, India

Mustafa Hussain, Policy and International Operations Regulatory, F. Hoffmann-La-Roche Ltd, Basel, Switzerland

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EXPERT INSIGHT

Translating both a cure and a commercialization pathway: insights into a breakthrough gene therapy company's journey

Daniel Drozdov and Nektarios Oraiopoulos

This article examines the commercialization pathway of Orchard Therapeutics (Orchard), tracing its evolution from an academic innovation hub to a biotech enterprise with an EMA- and US FDA-approved medicine. We analyze critical inflection points, from the 2018 acquisition of GSK's rare disease gene therapy portfolio to Orchard's acquisition by Kyowa Kirin and the FDA approval of Lenmeldy in 2024.

Drawing on extensive interviews with key decision makers, we show how Orchard addressed regulatory, manufacturing, and reimbursement hurdles by forging external production partnerships, crafting innovative pricing models, and shaping newborn screening policy. The case offers transferable lessons for scaling operations, aligning incentives, and accelerating market access.

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INTRODUCTION

The emergence of cell and gene therapies (CGTs) has reshaped treatment paradigms for rare diseases, offering curative potential in conditions long deemed untreatable. Yet, these advances come with considerable scientific, manufacturing, regulatory, and economic complexity.

The CGT sector has seen dramatic growth, with over USD 38 billion in acquisitions and USD 16 billion in licensing deals over the past decade. However, the pathway to market remains full of challenges, manufacturing difficulties, supply chain fragility, reimbursement obstacles, and access constraints. Expert consensus underscores the need for strategic market-entry plans, novel pricing models, and improved infrastructure to ensure enduring patient access [1]. Analysts forecast rapid expansion: the global CGT market size was estimated at USD 7.79 billion in 2024 and is predicted to increase from USD 8.94 billion in 2025 to approximately USD 39.61 billion by 2034, expanding at a Compound Annual Growth Rate of 17.98% from 2025–2034 [2].

Orchard Therapeutics (Orchard) presents a compelling study in strategic decision-making as it successfully navigated the challenges in the journey from an academic-rooted startup into a commercial-stage biotech firm with an EMA- and FDA-approved gene therapy product.

Drawing from extensive primary research and interviews with key stakeholders – including Orchard's leadership (Bobby Gaspar, Chief Executive Officer and Frank Thomas, Chief Operating Officer), former GSK executives, scientists, clinicians, market access experts, and patient advocates – this article provides insights and actionable lessons for biotech companies, healthcare policymakers, and investors.

Orchard Therapeutics was founded in 2015, but its transformative journey began in 2018, when it acquired GlaxoSmithKline's rare-disease gene therapy portfolio. The acquisition included Strimvelis (a pioneering EMA-approved therapy for Adenosine Deaminase-deficient Severe Combined Immunodeficiency (ADA-SCID) along with late-stage programs for metachromatic leukodystrophy (MLD), Wiskott–Aldrich syndrome (WAS), and beta-thalassemia, and licensing rights to preclinical programs in mucopolysaccharidosis type I (MPS-I), X-linked chronic granulomatous disease (X-CGD), and globoid cell leukodystrophy (GLD) from SR-Tiget. This bold move dramatically reoriented Orchard's capabilities and ambitions.

Under the leadership of CEO Bobby Gaspar, Orchard shifted from its early academic focus – developing lentiviral gene therapy for ADA-SCID in partnership with University College London (UCL) and University of California Los Angeles (UCLA) – to a comprehensive commercial model designed to deliver multiple therapies to market. Scientifically robust and supported by compelling clinical data, these programs nonetheless confronted formidable scaling and regulatory hurdles.

KEY CHALLENGES IN DEVELOPING AND COMMERCIALIZING GENE THERAPIES

Conducting multiple concurrent trials had required substantial capital, which compelled Orchard to align its portfolio with economic realities. Those clinical trials had to comply with stringent FDA and EMA requirements for well-powered clinical evidence, an especially daunting task given the small and heterogeneous patient pools in rare disease research. MLD has a birth prevalence from 0.16–1.85 per 100,000 live births [3]. Moreover, scaling lentiviral vector manufacturing proved a significant obstacle – as *ex vivo* gene therapies demanded high-quality production infrastructure, rigorous compliance systems, and costly external partnerships.

Determining appropriate pricing strategies became critical: therapies had to generate sufficient revenue to sustain operations, support future Research and Development (R&D), and satisfy payer constraints without compromising access.

Market success also hinged on robust stakeholder communication. Orchard prioritized therapies for conditions with acute unmet need – such as MLD – to strengthen its value proposition and attain broad access and reimbursement. Recognizing the importance of timely intervention, the company actively championed universal newborn screening – a strategic move that aligned patient outcomes, payer value, and policy reform.

CRAFTING A COMMERCIALIZATION STRATEGY

> "Gene therapy works because if it didn't, I would be dead."
>
> – 14-year old patient

MLD is a rapidly progressive neurodegenerative disorder resulting from ARSA gene

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mutations, characterized by sulfatide accumulation and eventual loss of motor and cognitive abilities. Without intervention, patients enter a vegetative state and die prematurely. Standard of care – supportive treatment alone – does not alter disease progression but incurs high lifelong costs: hospitalizations, adaptive devices, physiotherapy, and continuous caregiving [4,5].

Health economic modeling by Francis Pang, an employee of Orchard, revealed a lifetime cost of USD 6.6–10 million per patient in the US, factoring in medical care, lost productivity, and social burden. In contrast, MLD gene therapy – delivered pre-symptomatically – can potentially halt or slow disease progression and enable increased lifespan and function compared to no treatment, delivering an additional around 30 Quality-Adjusted Life Years (QALYs) per patient [6,7]. This translates to healthcare cost savings of approximately USD 5–7 million per patient, and it also eases caregiver burden by over 15 hours daily, preserving parental employment and quality of life.

Unlike traditional pharmaceuticals, CGTs demand a re-engineered value chain which often requires significant infrastructure investments. Orchard's approach was that of a network orchestrator model: leveraging rather than building infrastructure. Orchard's ecosystem orchestration is depicted in **Figure 1**.

Orchard's strength lay in its ability to orchestrate value rather than own it. First, outsourcing lentiviral vector production to AGC Biologics enabled Orchard to focus on product development and market access without the capital intensity of in-house manufacturing. The company shut down its California operations in 2020 to consolidate its workforce and extend financial runway.

From a pricing standpoint, Lenmeldy priced at over USD 4 million per patient, justifying the cost required robust QALY analyses and alignment with health technology assessment bodies. In Germany and the U.K., cost-effectiveness modeling helped secure early reimbursement.

The company benefited from proximity to SR-Tiget's research pipeline and built commercial momentum through selected partnerships. Notably, its partnership with MolMed (later acquired by AGC Biologics) ensured quality manufacturing capacity near the original R&D centers in Milan.

Strategically, Orchard embedded itself in newborn screening policy discussions, advocating for early diagnosis of MLD. In addition to advocacy from the MLD community, it lobbied for inclusion of MLD in national screening panels. This early detection infrastructure is vital since Lenmeldy is most efficacious in pre-symptomatic patients.

Stakeholder dynamics were integral to Orchard's success:

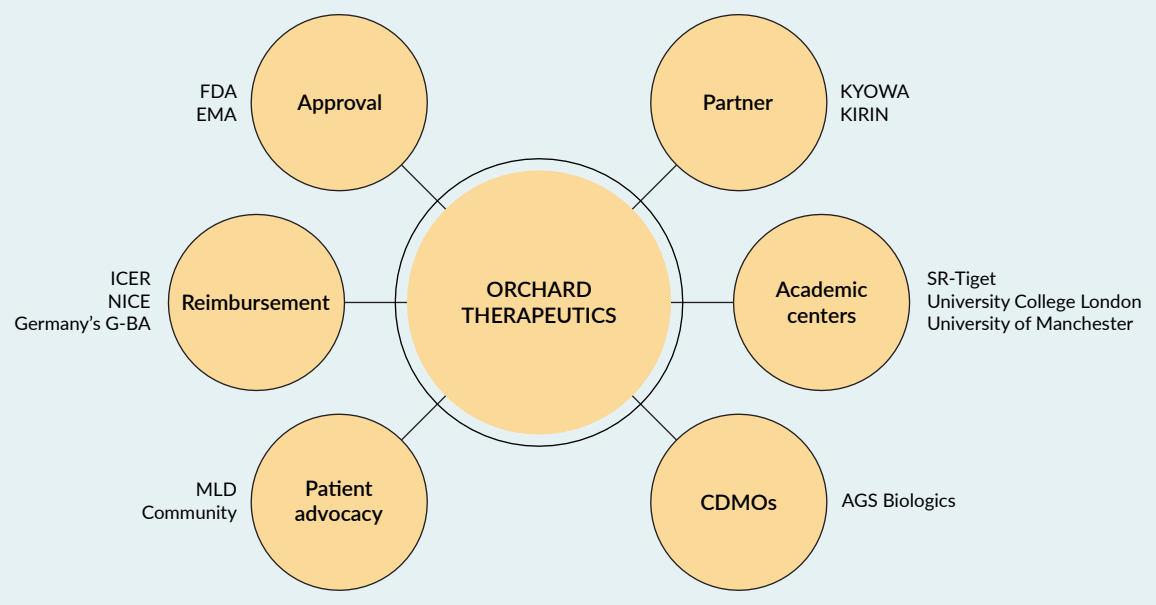
- Regulators demanded stringent CMC and long-term clinical follow-up (up to 15 years post-therapy).
- Payers needed compelling real-world data to justify one-time high-cost therapies.
- Families and patient groups acted as policy influencers and legitimacy builders.
- Investors sought capital discipline amidst a volatile biotech funding cycle.

By May 2022, the scale of Orchard's ambitions exceeded the resources available to sustain them. The company was running parallel programs across six indications but faced investor pressure to streamline. CEO Bobby Gaspar and COO Frank Thomas implemented a dramatic pivot:

- Prioritized neurometabolic disorders – especially MLD and MPSI – where no or limited alternative therapies existed

FIGURE 1

Orchard Therapeutics' ecosystem orchestration.



Central node: Orchard Therapeutics – the hub coordinating all activities. **Satellite nodes:** academic centers (e.g., SR-Tiget, University College London, University of Manchester) – innovation, research pipeline, early clinical data; contract development and manufacturing organizations (CDMOs) e.g., AGC Biologics – outsourced manufacturing of lentiviral vectors, drug product manufacturing, quality/compliance, cost efficiency; MLD community – policy support, newborn screening, real-world impact; reimbursement and health technology assessment (HTA) bodies (e.g., NICE, G-BA, ICER) – payer engagement, market access decisions; approval bodies (e.g., FDA, EMA).

and the clinical and economic value proposition was strongest.

- Discontinued and sought strategic alternatives for programs in ADA-SCID, WAS, and X-CGD, where existing treatments such as hematopoietic cell transplantation could compete.

- Reduced workforce by 30% to extend capital runway and focus on commercialization of the lead asset.

This shift required tough leadership decisions, including cutting programs that were once central to Orchard's pipeline. Bobby Gaspar emphasized that Orchard needed to be a leaner, more focused company, dedicated to delivering on the most impactful therapies.

This realignment extended Orchard's financial runway into 2024 and accelerated its path to commercial growth in Europe and sharpened its executional focus on regulatory submission for atidarsagene autotemcel in the US, even as the biotech sector faced a market downturn. These changes are reflected in the current pipeline, see Figure 2.

CGTs' promise hinges on their ability to address previously untreatable conditions using precision-engineered cells and vectors. Yet bringing these treatments to patients requires reimagining go-to-market strategies:

- Specialized treatment sites, trained providers, and intensive support are mandatory. For example, to ensure consistency and quality of treatment, only 5 centers in the US and 6 in Europe were initially qualified to administer MLD gene therapy as of January 2025 (see Figure 3).

- Autologous manufacturing creates scaling and affordability challenges. Allogeneic 'off-the-shelf' therapies may improve scalability, but regulatory complexity persists.

- Partnerships with CDMOs, such as MolMed/ AGC Biologics near SR-Tiget in Milan, enabled Orchard to rationalize operations and close the manufacturing facility in California, thereby avoiding the burden of carrying the CAPEX and OPEX associated with internal GMP operations.

Partnerships with large pharma were instrumental in Orchard's success. Orchard leveraged its tie to GSK and was itself later acquired by Kyowa Kirin ahead of FDA approval [8,9]. Similarly, CART developers like Novartis (Kymriah) and Gilead (Yescarta) pursued alliances or acquisitions for commercialization. By contrast, independent commercialization in this space by small biotechs has often faltered. Dendreon (Provenge) and Bluebird Bio (Zynteglo, Lyfgenia, Skysona) illustrate the pitfalls: manufacturing setbacks, rejection by European payers, and insufficient US uptake led to EU withdrawal and financial distress [1,10].

The commercial validation of Orchard's strategy came with EMA approval of atidarsagene autotemcel in 2020, followed by its FDA approval as Lenmeldy™ in 2024 post-acquisition by Kyowa Kirin. These approvals marked key inflection points, validating Orchard's progression from academic innovation to commercial execution.

LESSONS

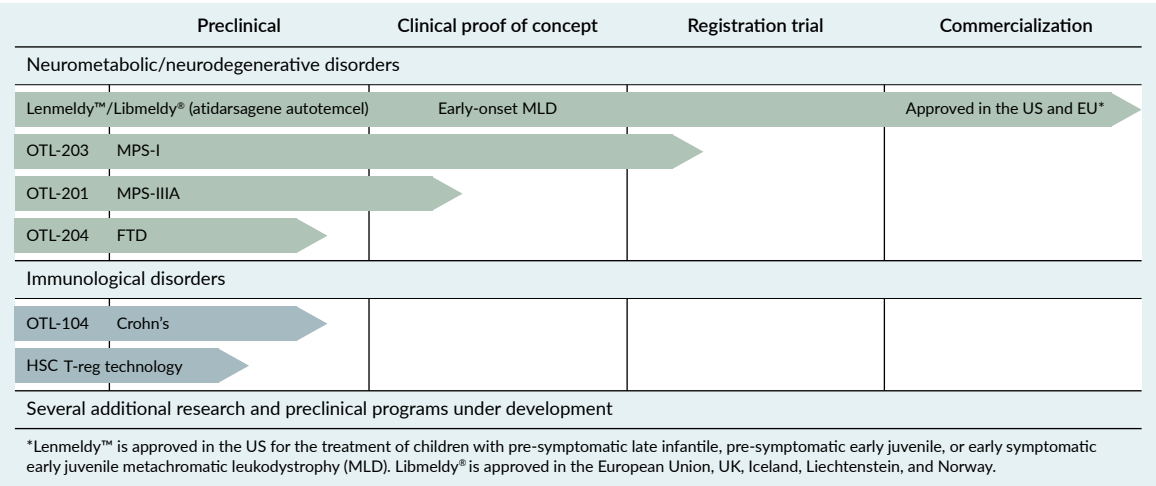
Orchard's journey offers a compelling blueprint for small and mid-sized CGT and Advanced Therapy Medicinal Products firms navigating a post-Initial Public Offering funding drought.

Be ready to make hard decisions to focus your R&D portfolio

By deliberately narrowing its pipeline at the May 2022 pivot, Orchard prioritized neurometabolic diseases – like MLD and MPS-I – with limited or no effective alternatives.

FIGURE 2

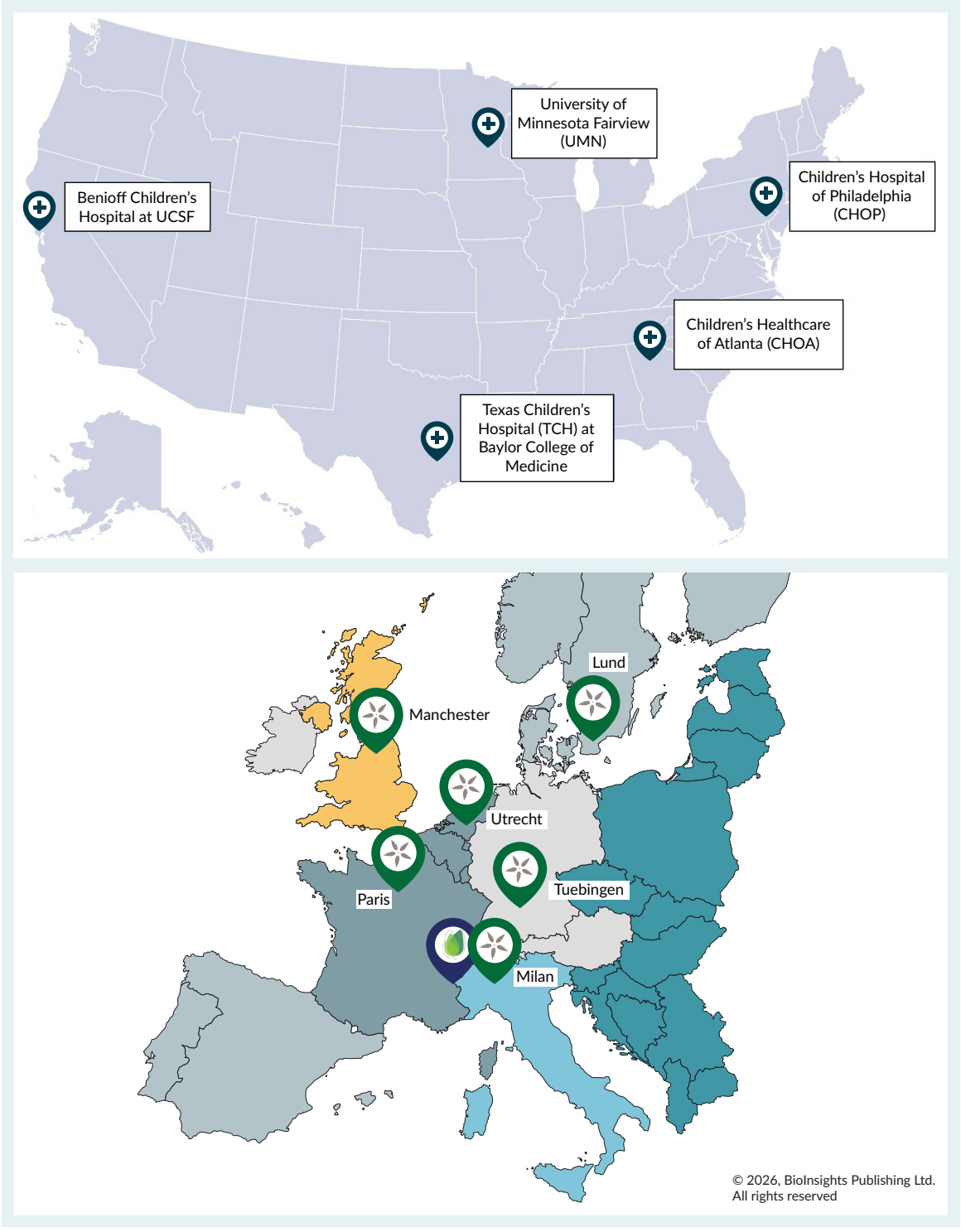
Orchard Therapeutics pipeline.



MLD: metachromatic leukodystrophy. MPS-IH: mucopolysaccharidosis type 1 – Hurler. MPS-IIIA: mucopolysaccharidosis type 3A – Sanfilippo. FTD: frontotemporal dementia.

FIGURE 3

Qualified Treatment Centers (QTCs) for Libmeldy/ Lenmeldy in the USA and Europe.



This disciplined focus mitigated resource dilution and enabled concentrated clinical and commercial efforts. Similar strategic restraint has been recommended by sector experts, who emphasize the importance of 'minimum viable product (MVP) that delivers clear value to early adopters' in funding-constrained environments.

Use academic and other partners to minimize capital burden

Orchard leveraged academic and manufacturing partnerships – such as with SR-Tiget and MolMed/AGC Biologics in Milan – to avoid building its own manufacturing infrastructure. This approach mirrors best practices in CGT, underscoring the value of using external capacity to maintain capital efficiency while scaling.

This outward looking approach was critical throughout Orchards' journey: rather than becoming overly dependent on high-risk stand-alone commercialization, Orchard maintained flexibility for strategic partnership or acquisition. This allowed it to transition into Kyowa Kirin while preserving its mission and team – providing a tailored exit aligned with long-term value creation.

ALIGN PRICING WITH MEASURABLE LONG-TERM OUTCOMES

Orchard invested early in cost-effectiveness modeling, demonstrating that gene therapy has the potential to deliver more than 30 QALYs per patient and lifetime savings of approximately USD 5–7 million – well within conventional cost-effectiveness thresholds. Systematic evidence demonstrated long-term healthcare cost savings and quality-of-life gains and lent credibility to Lenmeldy's USD 3 to 4 million price tag [6]. This aligns with advisory frameworks calling for 'compelling data, realistic timelines, and holistic value propositions.' Establishing this economic narrative in advance strengthened Orchard's reimbursement positioning.

Treat patient advocacy as part of the go-to-market strategy

Recognizing the importance of market access, Orchard engaged payers well before launch and advocated to include MLD in newborn screening panels.

Gene therapies demand novel commercial architectures – including specialized treatment centers, provider training, and patient support. Orchard demonstrated this by building infrastructure for MLD delivery and advocating for newborn screening policy development. Its pricing strategy for Lenmeldy reflected a balance between therapeutic value and payer acceptance. This long-lead outreach ensured both eligibility and reimbursement readiness – differentiating Orchard from peers who encountered delayed access due to limited stakeholder preparation.

CONCLUSION

Orchard Therapeutics' transition from a translational research spinout to a commercial entity with an EMA- and FDA-approved medicine was far from a linear trajectory. Its success reflects an intentional design of partnerships, pricing models, policy engagement, and strategic restraint. In doing so, it not only brought life-altering therapies to patients but also pioneered a business model fit for the next generation of high-impact biomedicine.

CGT therapy, once a scientific aspiration, now hinges on executional excellence. Orchard's case suggests that impact and sustainability require more than technological promise: they demand system-level thinking, aligned incentives, and the discipline to say no.

The commercialization journey of Orchard Therapeutics offers critical insights into the operational, financial,

and strategic complexities inherent in the biotech industry.

Orchard's experience highlights that scientific breakthroughs alone are insufficient to secure market viability. Success requires aligning manufacturing prowess, regulatory know-how, reimbursement strategies, and focused market position.

These lessons are broadly applicable to emerging biotech companies, particularly in the CGT space. In an industry marked by funding scarcity and regulatory complexity, strategic clarity, economic rigor, proactive stakeholder alignment, and flexible operational models are essential to transform scientific innovation into sustainable therapeutic impact.

In sum, Orchard Therapeutics exemplifies how advanced therapies can be commercialized sustainably. For biotechnology leaders, investors, and policymakers, Orchard's trajectory serves as a blueprint: to bring transformative therapies to patients, one must design an ecosystem that balances innovation with pragmatic execution.

REFERENCES

1. Piñel-Neparidze C, Bickerstaffe H, Shah S, Versnel J. The importance of a go-to-market strategy in the commercialisation of cellular immunotherapies. *Drug Discov. Today* 2024; 29, 104028.
2. Suryaprakash S, Teixeira A, Choy M. The Changing Landscape for Cell and Gene Therapy. *Boston Consulting Group.* May 5, 2021. https://mkt-bcg-com-public-pdfs.s3.amazonaws.com/prod/understanding-the-rapidly-changing-cell-and-gene-therapy-landscape.pdf.
3. Chang S-C, Bergamasco A, Bonnin M, Bisonó TA, Moride Y. A systematic review on the birth prevalence of metachromatic leukodystrophy. *Orphanet J. Rare Dis.* 2024; 19, 80.
4. Kefalas MJ. *Harnessing Grief: A Mother's Quest for Meaning and Miracles.* 2021; Beacon Press.
5. Armstrong N, Olaye A, Noake C, Pang F. A systematic review of clinical effectiveness and safety for historical and current treatment options for metachromatic leukodystrophy in children, including atidarsagene autotemcel. *Orphanet J. Rare Dis.* 2023; 18, 248.
6. Pang F, Dean R, Jensen I, Bean K, Fields C, Miller B. The cost-effectiveness of OTL-200 for the treatment of metachromatic leukodystrophy (MLD) in the US. *Mol. Genet. Metab.* 2023; 138, 107258.
7. Fumagalli F, Calbi V, Natali Sora MG, *et al.* Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: long-term results from a non-randomised, open-label, Phase 1/2 trial and expanded access. *Lancet* 2022; 399, 372–383.
8. Taylor NP. Orchard licenses gene therapy tech from GSK. *FierceBiotech.* Jul 15, 2020. https://www.fiercebiotech.com/biotech/orchard-licenses-gene-therapy-tech-from-gsk.
9. Jensen K. Orchard follows buyout with FDA approval of rare disease gene therapy. *BioPharma Dive.* Mar 19, 2024. https://www.biopharmadive.com/news/orchard-fda-approval-lenmeldy-gene-therapy-metachromatic-leukodystrophy/710673.
10. Peebles A. Once high-flying Bluebird Bio sells itself to private equity after tough times for the gene therapy maker. *CNBC.* Feb 21, 2025.https://www.cnbc.com/2025/02/21/bluebird-bio-gene-therapy-sells-itself-to-carlyle-and-sk-capital.

AFFILIATIONS

Daniel Drozdov, Children's Hospital, Kantonsspital Aarau, Aarau, Switzerland and University Children's Hospital Zurich, Zurich, Switzerland

Nektarios Oraiopoulos, Cambridge Judge Business School, University of Cambridge, Cambridge, UK

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INDUSTRY INSIGHTS • JANUARY 2026

From rare disease to chronic indications, cell and gene therapies broaden their impact

Abi Pinchbeck

As a commissioning editor with extensive experience in advanced therapy journal publishing, Abi's focus is on progressing the field by facilitating and disseminating high-impact, open access content covering novel and existing cell and gene therapies. Abi works closely with academic scientists and industry professionals to publish cutting-edge original research, expert reviews, and multimedia content with a translational and interdisciplinary focus. Abi's key aim is to explore the latest advances in cell and gene therapy R&D, clinical development, manufacturing, and commercialization. In addition to Abi's editorial responsibilities, she maintains a strong network of experts across the biotech and pharma industries, staying up to date with emerging trends and breakthroughs in advanced therapies.

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SUMMARY

Wrapping up 2025 and commencing 2026, this edition highlights the continued expansion of cell and gene therapies beyond ultra-rare indications into chronic, degenerative, and inflammatory diseases, underpinned by advances in clinical and manufacturing maturity. Momentum continued across autologous and allogeneic cell therapies, including Parkinson's and Duchenne muscular dystrophy programs, alongside efforts to extend engineered cell modalities beyond oncology, with CAR-Tregs explored in cardiovascular inflammation and CAR-NKT positioned as an off-the-shelf approach for solid tumors. US FDA actions reflected both expansion and caution, with approvals broadening access to gene replacement therapies alongside ongoing efforts to address AAV-associated safety risks. Ophthalmology emerged as a key focus, spanning first-in-human bioprinted corneal implantation, stem cell-derived retinal therapies in dry age-related macular degeneration (AMD), and a partnership targeting intravitreal AAV delivery for intermediate AMD. Business activity remains agile, with some promising financings, newco launches, and an acquisition centered on lipid nanoparticle delivery platforms.

COMPANY START-UPS

Link Cell Therapies launched from stealth with a $60 million Series A to advance logic-gated CAR-T programs [1]

Link Cell Therapies launched from stealth and reported a $60 million Series A financing led by Johnson & Johnson Innovation – JJDC, Inc., with participation from Samsara BioCapital, Sheatree Capital, Wing Venture Capital, and strategic investors including Bristol Myers Squibb and Kyowa Kirin. Link said it had raised $92 million across seed and Series A rounds. The company stated its lead program, LNK001 in renal cell carcinoma, was on track for an investigational drug application and Phase 1 dosing in 2026, with a colorectal cancer program targeting development candidate selection in 2026 and human studies in 2027 [1].

Addition Therapeutics emerged from stealth with $100 million to develop LNP-delivered RNA-to-DNA genetic medicines [2]

Addition Therapeutics emerged from stealth with $100 million to develop gene therapies intended to address limitations of viral vector delivery and durability. The company described a lipid nanoparticle (LNP) platform delivering RNA-to-DNA cargo targeted to specific genomic regions, and cited applicability across rare and chronic diseases, including an HIV program in collaboration with the Gates Foundation designed to drive long-term expression of protective antibodies.

REGULATORY UPDATES

Novartis received US FDA approval for Itvisma, an intrathecal gene replacement therapy for SMA in older patients [3]

Novartis reported US FDA approval of Itvisma, described as an intrathecal version of onasemnogene abeparvovec (Zolgensma), expanding gene replacement therapy access to patients with spinal muscular atrophy (SMA) beyond infants. The company stated it planned an imminent launch and listed a $2.59 million price. Novartis said the approval was supported by Phase 3 data showing improved and stabilized motor function. The intrathecal route was positioned to enable dosing in children aged 2 years and older, as well as teens and adults.

Sarepta received US FDA clearance to start ENDEAVOR Cohort 8 to evaluate a sirolimus-based immunosuppression regimen with ELEVIDYS [4]

Sarepta announced US FDA clearance to initiate Cohort 8 of the ENDEAVOR study to evaluate an enhanced sirolimus-based immunosuppression regimen intended to reduce acute liver injury and acute liver failure associated with AAV gene therapy in non-ambulatory Duchenne muscular dystrophy patients receiving ELEVIDYS. The company said ~25 non-ambulatory participants were expected to receive sirolimus as

part of the regimen, and that Cohort 8 was expected to begin before year-end. Sarepta added that decisions on resuming commercial dosing for this population would be made in collaboration with the FDA after reviewing Cohort 8 data.

The US FDA approved Waskyra, an *ex vivo* lentiviral HSC gene therapy for Wiskott–Aldrich syndrome developed by a nonprofit [5]

A report described FDA approval of Fondazione Telethon ETS's etuvetidigene autotemcel (Waskyra) for Wiskott–Aldrich syndrome (WAS), characterizing it as the first US gene therapy approval led by a nonprofit organization and the first gene therapy approved for WAS. The *ex vivo* therapy involved harvesting patient hematopoietic stem cells (HSCs), engineering them with lentiviral vectors to add a functional *WAS* gene sequence, and reinfusing the modified cells following conditioning with rituximab, busulfan, and fludarabine. The approval was based on two open-label, single-arm trials in 27 patients, with reported reductions in severe infection rates (from 2.0 to 0.2 infections per patient-year) and moderate/severe bleeding events (from 2.0 to 0.8 events per patient-year) after treatment, with follow-up reported up to 13.3 years.

MARKET TRENDS

Aspen Neuroscience raised $115 million to advance its autologous Parkinson's cell therapy program [6]

Aspen Neuroscience reported the close of a $115 million Series C financing to support clinical development of ANPD001, an autologous induced pluripotent stem cell (iPSC)-derived dopaminergic neuronal precursor cell therapy for moderate to advanced Parkinson's disease. The round was co-led by OrbiMed, ARCH Venture Partners, Frazier Life Sciences, and Revelation Partners, with new participation including Kite, a Gilead company). Aspen said proceeds would support ongoing ANPD001 trials, manufacturing scale-up for clinical and commercial supply, and pipeline expansion into additional neurological indications. The company highlighted dosing in the Phase 1/2a ASPIRO trial Cohort 3 using a cryopreserved, ready-to-dose commercial formulation and noted FDA Fast Track designation for ANPD001.

XOMA Royalty agreed to acquire Generation Bio and add its cell-targeted LNP delivery platform to its portfolio [7]

XOMA Royalty announced an agreement to acquire Generation Bio for $4.2913 per share in cash, with Generation Bio stockholders also receiving a non-transferable contingent value right (CVR). The CVR was structured to provide potential payments tied to net cash at closing above $29 million, potential savings related to Generation Bio's Cambridge lease obligations, and proceeds from existing and future licensing of Generation Bio assets. XOMA said Generation Bio's cell-targeted LNP delivery platform for small interfering RNA and other nucleic acid therapies would be included in XOMA's portfolio. The CVR also provided for a share of proceeds from Generation Bio's collaboration with Moderna, including potential development and commercial milestones and royalties on sales, delivered on a sliding scale up to 90% to CVR holders.

RESEARCH AND DEVELOPMENT HIGHLIGHTS

Penn researchers engineered anti-oxLDL CAR-Tregs that reduced atherosclerotic plaque in mice [8]

Researchers at the University of Pennsylvania reported a preclinical approach using CAR-T cells built from regulatory T cells (Tregs) to suppress inflammation in atherosclerosis. The team engineered CAR-Tregs targeting oxidized LDL (OxLDL) and showed, in initial in vitro experiments with human cells, that the engineered cells suppressed inflammatory responses to OxLDL and reduced plaque-associated cell buildup. In a mouse model predisposed to hypercholesterolemia and atherosclerosis, treatment with a mouse anti-OxLDL CAR-Treg reduced atherosclerotic plaque burden by ~70% after ~12 weeks versus controls, without reported disruption of general immune function. The investigators and Penn formed a spinout, Cartio Therapeutics, to advance the program toward clinical testing.

UCLA researchers developed an off-the-shelf CAR-NKT approach targeting mesothelin in pancreatic cancer models [9]

UCLA researchers described a preclinical engineered cell therapy platform using invariant natural killer T (NKT) cells modified with CAR targeting mesothelin (CAR-NKT) for pancreatic cancer. The team reported that the cell product could be mass-produced from donated blood stem cells and stored as an off-the-shelf therapy, and cited an estimated cost of ~$5,000 per dose. In orthotopic and metastatic mouse models (including liver metastasis models), the CAR-NKT cells were reported to home to tumor sites, infiltrate tumors, slow tumor growth, and extend survival, with minimal exhaustion signals in the tested settings. The investigators stated they were preparing submissions to the FDA to initiate clinical trials.

CLINICAL TRIALS AND RESEARCH

Hope Biosciences reported Phase 2 data for an allogeneic adipose-derived MSC therapy in early to moderate Parkinson's disease [10]

Hope Biosciences Research Foundation reported positive topline results from a Phase 2 randomized, double-blind, placebo-controlled, single-center trial (NCT04995081) evaluating allogeneic adipose-derived mesenchymal stem cells (HB-adMSCs) in early to moderate Parkinson's disease. Sixty participants were enrolled (30 treatment; 30 placebo) and received six intravenous infusions of 200 million cells over 32 weeks, with end of study at 52 weeks. The trial met its primary endpoint, with statistically significant motor function

improvements versus placebo based on clinician-rated MDS-UPDRS Part III. By the sixth infusion, the treatment group achieved a mean change from baseline of −9.82 points versus −0.50 in placebo (adjusted mean difference −9.32; 95% CI [−15.11, −3.54]; p=0.0023). The organization reported the regimen was safe and tolerable.

Kyverna with positive registrational Phase 2 KYSA-8 data for miv-cel in stiff person syndrome [11]

Kyverna Therapeutics reported positive topline data from KYSA-8, a single-arm registrational Phase 2 study of mivocabtagene autoleucel (miv-cel; KYV-101), an autologous CD19-targeting CAR-T with CD28 co-stimulation, in stiff person syndrome. Twenty-six patients received a single dose and were followed through the week 16 primary analysis timepoint. The company reported statistically significant improvement in timed 25-foot walk (p=0.0002) with a median 46% improvement at week 16; 81% exceeded a 20% improvement threshold. Secondary endpoints (including modified Rankin Scale and stiffness and ambulation indices) were reported as highly significant (all p<0.0001), and 100% remained free of immunotherapies at last follow-up. Kyverna reported no high-grade cytokine release syndrome or immune effector cell-associated neurotoxicity syndrome and said it planned a BLA submission in 1H 2026.

Precise Bio dosed the first patient with a 3D-bioprinted corneal implant in a Phase 1 study [12]

Precise Bio announced the first successful human implantation of PB-001, a cell-based, 3D-bioprinted corneal implant manufactured from cultured human corneal cells. The procedure was performed on October 29, 2025, at Rambam Medical Center in an ongoing Phase 1, single-arm trial in patients with corneal edema due to endothelial dysfunction. The company said PB-001 was produced using a robotic 3D-bio-fabrication system at a GMP facility at Sheba Medical Center, and was designed for cryopreservation and use with standard delivery devices. The study planned to enroll 10–15 patients and evaluate safety and tolerability, with exploratory 6-month efficacy outcomes; topline results were expected in 2H 2026.

Michigan Medicine reported early Phase 1/2a data for adult stem cell-derived RPE transplants in advanced dry AMD [13]

Michigan Medicine researchers reported early findings from a Phase 1/2a study evaluating retinal pigment epithelial (RPE) stem cell transplants for advanced dry age-related macular degeneration (AMD). The RPE stem cells were derived from adult postmortem donor eye tissue and were described as lineage-restricted to mature into RPE cells. In the lowest-dose cohort, six participants received 50,000 cells during ocular surgery; the investigators reported no serious inflammation or tumor growth. The treated eyes showed vision improvements compared with untreated eyes, with the low-dose cohort reading 21 additional letters on a standard eye chart 1 year after treatment. The team was monitoring additional cohorts dosed at 150,000 and 250,000 cells and stated that later-stage clinical development would be considered if safety remained acceptable.

Capricor reported positive topline Phase 3 HOPE-3 results for deramiocel in Duchenne muscular dystrophy [14]

Capricor Therapeutics reported positive topline results from HOPE-3, a pivotal Phase 3 randomized, double-blind, placebo-controlled trial (n=106) evaluating

deramiocel, an investigational cell therapy, in Duchenne muscular dystrophy. The company said the study met the primary endpoint (PUL v2.0; p=0.03), and a key secondary cardiac endpoint (LVEF; p=0.04), with statistical significance achieved across all type 1 error-controlled secondary endpoints. Participants received intravenous deramiocel (150 million cells per infusion) or placebo every 3 months for 12 months. Capricor reported a safety and tolerability profile consistent with prior experience and said it planned to submit a response to a previously received Complete Response Letter incorporating HOPE-3 data.

First-in-human stem cell gene therapy shows early promise for Hunter syndrome [15]

Researchers at the University of Manchester have reported early clinical progress from a first-in-human stem cell gene therapy for Hunter syndrome, or mucopolysaccharidosis type II, following treatment of a 3-year-old patient at Royal Manchester Children's Hospital. The one-off autologous therapy involves *ex vivo* genetic modification of HSCs to restore production of the deficient enzyme, with the aim of achieving systemic and central nervous system delivery. Several months post-transplant, the patient has discontinued weekly enzyme replacement therapy and is showing sustained high circulating enzyme levels. The investigator-led study, sponsored by the University of Manchester, will enroll five children and builds on a decade of translational development. The approach could offer a safer, more effective alternative to donor bone marrow transplantation and has potential applicability across other inherited metabolic disorders.

COLLABORATIONS AND PARTNERSHIPS

Ikarovec optioned VectorBuilder's intravitreal AAV capsid technology for IKAR-003 in intermediate AMD [16]

Ikarovec and VectorBuilder announced an exclusive worldwide option agreement for VectorBuilder's AAV capsid technology to support Ikarovec's gene therapy candidate IKAR-003 for intermediate AMD. Ikarovec said that, following further evaluation, the parties would enter a strategic partnership in which Ikarovec would lead clinical development and commercialization, with the proposed deal expected to be worth over $1 billion. The companies stated the capsid was intended to enable intravitreal delivery of IKAR-003, a one-time dual-pathway gene therapy combining neuroprotection and complement modulation to prevent progression to geographic atrophy or wet AMD. VectorBuilder cited non-human primate data indicating broad retinal transduction via intravitreal administration, including macular cell targeting.

REFERENCES

1. Link cell therapies launches with vision of advancing CAR-T therapies in solid and liquid tumors. *PR Newswire.* Dec 15, 2025.
2. Wu G. Addition emerges with $100M to make gene therapies for chronic and rare diseases. *BioPharma Dive*. Dec 17, 2025.

3. Novartis. Novartis receives FDA approval for Itvisma®, the only gene replacement therapy for children two years and older, teens, and adults with spinal muscular atrophy (SMA). Nov 24, 2025.
4. Sarepta. Sarepta announces approval to begin ENDEAVOR cohort 8 to evaluate enhanced immunosuppression regimen as part of ELEVIDYS gene therapy for non-ambulant individuals with Duchenne. Nov 25, 2025.
5. Mullard A. FDA approves first gene therapy from a non-profit organization, but pricing and access questions remain. *Nat. Rev.* Dec 17, 2025.
6. Incorvaia D. Aspen enriches Parkinson's cell therapy trial with Kite-backed $115M series C. *Fierce Biotech* Nov 20, 2025.
7. XOMA Royalty. XOMA Royalty enters into agreement to acquire Generation Bio. Dec 15, 2025.
8. Penn Medicine. New CAR T strategy targets most common form of heart disease. Nov 21, 2025.
9. Wang L. UCLA scientists develop one-product-fits-all immunotherapy for pancreatic cancer. *UCLA Health* Nov 24, 2025.
10. Hope Biosciences Research Foundation. Hope Biosciences Research Foundation Reports Promising Phase II Trial Results for Stem Cell Therapy in Parkinson's Disease. *Business Wire* Dec 22, 2025.
11. Kyverna Therapeutics. Kyverna Therapeutics announces positive topline data from registrational KYSA-8 trial of miv-cel (KYV-101) in stiff person syndrome. Dec 15, 2025.
12. Precise Bio. Precise Bio Achieves World's First 3D-Bio-Printed Corneal Implant. *BioSpace* Nov 20, 2025.
13. Michigan Medicine – University of Michigan. Stem cell therapy helps AMD patients see again. *ScienceDaily* Nov 22, 2025.
14. Capricor Therapeutics. Capricor Therapeutics announces positive topline results from pivotal Phase 3 HOPE-3 study of deramiocel in Duchenne muscular dystrophy. Dec 3, 2025.
15. University of Manchester. New hope for children with devastating rare genetic disorder, thanks to world-first research in Manchester. Nov 24, 2025.
16. Ikarovec. Ikarovec to advance pipeline with exclusive worldwide option to VectorBuilder's intravitreal capsid technology. Jan 6, 2026.

INNOVATOR INSIGHT

Scaling AAV5 production from bench to industrial scale: strategies for efficient, de-risked manufacturing

Sara Krekels and Anne MacIntyre

Gene therapy is progressing quickly, but persistent manufacturing challenges still restrict its accessibility to reach patients in need. This article outlines how these barriers can be addressed by implementing more efficient, scalable production platforms. To overcome production limitations, advanced manufacturing systems can provide an integrated approach designed to improve efficiency, consistency, and scalability. The case study in this article illustrates how an advanced platform supports robust AAV5 production in adherent cells, presenting comparative data across three fixed-bed bioreactor scales. The results demonstrate a complete and practical scaling strategy from benchtop to large-scale manufacturing, highlighting the potential of advanced bioreactor systems to streamline gene therapy production and expand patient access.

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OVERVIEW OF GENE THERAPY'S PROGRESS, CHALLENGES, & ECONOMIC CONSIDERATIONS

Gene therapy is being recognized as a transformative force in healthcare. Since 2022, the pace of US FDA approvals has accelerated, with therapies such as LUXTURNA™, ZOLGENSMA®, and ELEVIDYS® paving the way. In particular, AAV vectors have been established as a leading platform for the *in vivo* delivery of therapeutic genes due to their safety and effectiveness. As a result, these therapies are now offering hope for conditions that were previously considered untreatable.

While the promise of gene therapy is evident, cost remains a significant barrier. Recent approvals have demonstrated prices of approximately $2.5–$3.5 million per dose. When compared with the lifetime cost of conventional treatment, where such treatment exists, these prices are often similar or lower. However, the current healthcare system is not structured to manage such substantial upfront costs, which has limited widespread access to these potentially curative therapies, particularly for ultra-rare diseases. Reducing

production costs is therefore essential in order to improve accessibility.

TRANSITIONING FROM TRADITIONAL FLATWARE PRODUCTION TO SCALABLE, AUTOMATED MANUFACTURING

The traditional upstream production method for viral vector-based therapies has relied on 2D flatware for cell growth and vector manufacturing. These processes require very large facilities to accommodate numerous multi-tray incubators, each of which must be seeded, transfected, and harvested individually. The approach is highly labor intensive, and every manual intervention introduces a potential contamination risk. In addition, cell cultures are difficult to monitor or control, which often results in substantial and poorly understood batch-to-batch variability. Further scale-up using this format is often not practical.

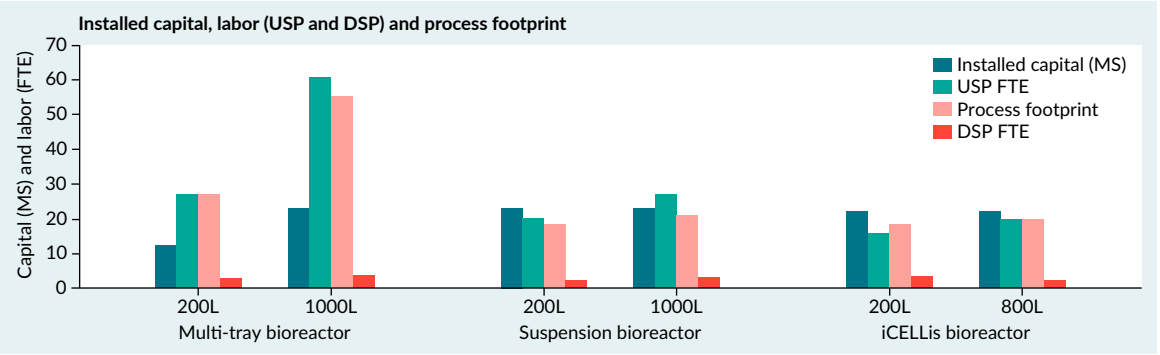
Advanced therapies can be produced more efficiently using single-use bioreactors. When compared with multi-tray platforms, bioreactors markedly reduce labor and space requirements while maintaining comparable capital costs . In addition, they enable fully closed process handling, batch automation, and live culture monitoring, which results in reduced variability, faster scale-up, and lower operational complexity.

Advanced systems, such as iCELLis™ technology from Cytiva, can be utilized for adherent cell and gene therapy manufacturing. The iCELLis bioreactors are equipped with GMP-compatible (21 CFR Part 11) software that enables process automation and incorporates integrated sensors (pH, dissolved oxygen (DO), temperature, and biomass) for real-time culture monitoring. Additionally, the platform enables scalability, beginning at 0.5 m^2 of cell growth surface area and extending to 500 m^2. The system is also widely used in clinical trial manufacturing and has already been applied in the production of six approved, commercially available therapeutics.

The iCELLis family includes three different bioreactor scales: iCELLis Nano, iCELLis 50, and iCELLis 500+. The benchtop-scale iCELLis Nano bioreactor provides 0.5–4 m^2 of cell growth surface area, the fully single-use iCELLis 50 bioreactor 6.6–50 m^2, and the industrial-scale iCELLis 500+ bioreactor 66–500 m^2. Together, these systems enable the iCELLis technology to support preclinical development, clinical trial manufacturing, validation runs, and full-scale commercial production.

FIGURE 1

Comparison of cost and labor requirements for upstream and downstream cell and gene therapy processing using multi-tray platforms, suspension bioreactors, and iCELLis fixed-bed adherent bioreactors.



Efficient scaling of gene therapy production requires movement beyond traditional manufacturing methods, and advanced technologies such as the iCELLis bioreactors can provide a scalable approach, designed to reduce production costs and operational complexity.

CASE STUDY: UTILIZING iCELLis BIOREACTOR TECHNOLOGY FOR SCALABLE AAV5 PRODUCTION

In a proprietary study, AAV5 production was performed across all three iCELLis bioreactor scales. Each bioreactor vessel used during testing was configured with low carrier compaction and a fixed bed height of 10 cm, resulting in cell growth surface areas of 2.65 m^2 for the iCELLis Nano bioreactor, 33 m^2 for the iCELLis 50 bioreactor, and 333 m^2 for the iCELLis 500+ bioreactor.

Several culture rounds were conducted, with multiple scales operated in parallel whenever possible. In total, six runs were completed using the iCELLis Nano bioreactor, three runs were completed using the iCELLis 50 bioreactor, and one run was performed using the iCELLis 500+ bioreactor. Each bioreactor was inoculated at a target density of 5,000 cells/cm^2, followed by 5 days of cell growth in a serum-containing medium.

Because the required total media volume exceeded the capacity of the bioreactor vessel, an external biocontainer was used to hold the remaining media, which was automatically recirculated in and out of the vessel throughout the growth phase. Immediately before transfection on day 5, a complete media exchange was performed to replace the serum-containing medium with serum-free medium. Triple transfection was then carried out using a complex containing 0.2 µg total plasmid DNA/cm^2 of cell growth area together with the transfection reagent PEI MAX.

Transfection was followed by 5 additional days of culture for AAV5 production in serum-free medium, again with recirculation. Harvesting included the collection of the spent supernatant, the lysate generated from an overnight lysis of the cells within the vessel, and a final PBS wash fraction. Collected samples were frozen and later analyzed for genomic titer and capsid titer by ddPCR and ELISA, respectively.

Because all three iCELLis scales were used, several key variables were considered to ensure consistent execution of the AAV process across scales. To reduce variability in input materials during parallel runs, all solutions, including media, transfection complex, and lysis buffer, were prepared as single batches and divided across scales immediately before use. For example, one transfection complex was prepared and incubated for 15 min. Afterwards, a defined portion was used for transfection of the iCELLis Nano systems, and the remaining defined volume was used for the iCELLis 50 or iCELLis 500+ vessels.

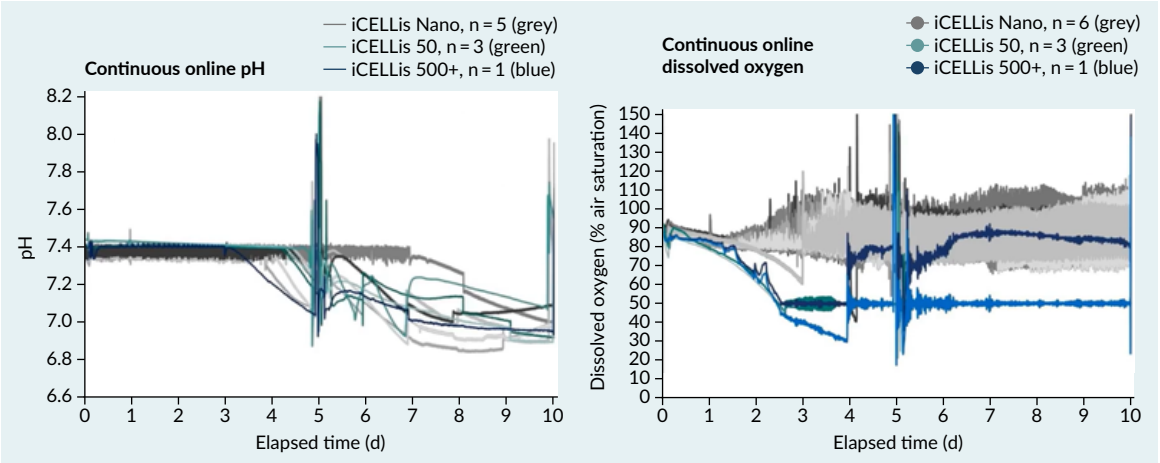
The linear speed of media flowing upward through the fixed bed, along with the ratio of media volume to cell growth surface area, can be critical for maintaining a homogeneous environment that supports consistent cell growth and productivity. The falling-film height, which describes the height of the downward-flowing media film, can influence the efficiency of low-shear gas exchange. Parameter selection for these variables should align with the priorities of the targeted process step.

EVALUATING pH & DO TRENDS IN CASE STUDY iCELLis BIOREACTORS

Based on the cell cultures conducted, the pH trends were similar across all three culture scales – iCELLis Nano, iCELLis 50, and iCELLis 500+ bioreactors . Control was maintained at the high end

FIGURE 2

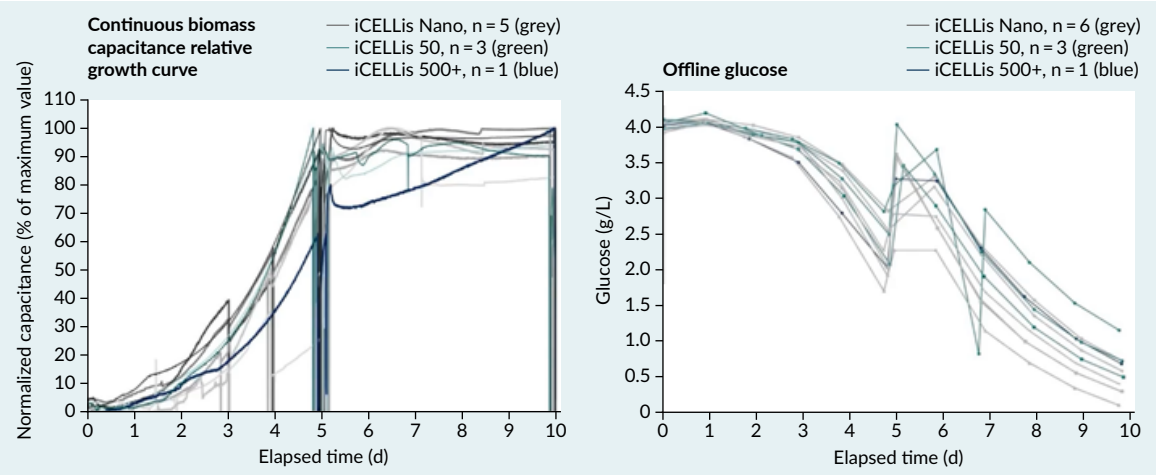
Examination of continuous pH (A) and dissolved oxygen (B) trends across all iCELLis bioreactor scales.



iCELLis Nano bioreactor runs appear in varying shades of gray, the iCELLis 50 bioreactor runs in green, and the iCELLis 500+ bioreactor runs in blue. (A) A brief deviation is visible around days 6 and 7 in one of the iCELLis 50 bioreactor runs, caused by an overnight recirculation malfunction. (B) DO set points differ across scales.

FIGURE 3

Trends in biomass capacitance and glucose concentration.



(A) Biomass capacitance trends, providing real-time cell growth monitoring across scales. Capacitance values were normalized to each batch's maximum, with spikes resulting from planned agitation stoppages during sampling and media exchanges. (B) Glucose concentration trends. Glucose is shown as a representative metabolite, as all measured metabolites displayed consistent patterns across scales, demonstrating effective process scalability. A brief deviation is visible around days six and seven in one of the iCELLis 50 bioreactor runs, caused by an overnight recirculation malfunction.

of the pH deadband during most of the cell growth phase, followed by a steady decline in pH immediately before and after the media exchange and transfection on day 5. Overall, the pH profiles were aligned within the expected variability across scales.

For DO control, the iCELLis Nano bioreactor was operated at a set point of 80%, while the iCELLis 50 and iCELLis 500+

bioreactors were operated at a lower set point of 50%, owing to the positioning of their DO sensors relative to the fixed-bed structure . After a DO control correction was applied on day 4 in the iCELLis 500+ bioreactor, sensor measurements taken after the fixed bed (light blue lines) closely resembled those from the iCELLis 50 bioreactor, while measurements taken before the fixed bed (dark blue line) aligned with those from the iCELLis Nano. bioreactor. This pattern is consistent with the expected control strategy and reflects the influence of cellular oxygen consumption within the fixed bed.

In summary, pH trends were comparable across all scales, whereas DO profiles differed because each system required its dedicated control approach.

ASSESSING BIOMASS GROWTH PATTERNS & METABOLITE CONSISTENCY ACROSS iCELLis BIOREACTOR SCALES

Bioreactor biomass analysis showed a consistent increase in capacitance during the cell growth phase, followed by a plateau in values after transfection .

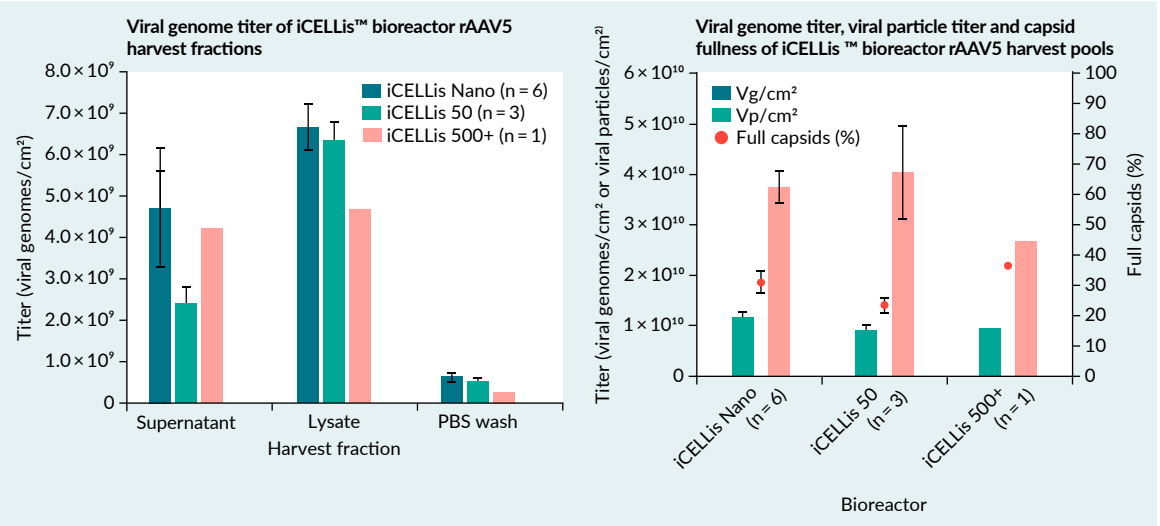
Glucose trends also served as an indirect indicator of cell growth for each run . Fixed-bed carriers from the iCELLis Nano bioreactor were sampled prior to transfection, with day-5 cell counts ranging between 117,000 and 129,000 cells/cm² across production runs. The uniformity of the glucose profiles, together with the tight cell count range observed in the iCELLis Nano bioreactors, confirmed that cell densities were comparable across all systems at the time of transfection.

EVALUATING HARVEST YIELDS, VIRAL TITERS, & CAPSID CHARACTERISTICS ACROSS iCELLis BIOREACTOR SCALES

As noted earlier, the bioreactors were harvested after a 5-day production phase. The spent-media supernatant, cell-lysate fraction, and PBS-wash fraction were collected and combined to generate the final harvest pool per each culture. Harvest fractions were analyzed for genomic titer only,

FIGURE 4

Viral genome titers and capsid fullness across harvest fractions and bioreactor scales.



(A) Viral genome titers measured in each harvest fraction. (B) Total viral particles and viral genomes in the pooled harvest for all bioreactor scales. Full capsid percentages were calculated directly from viral genome and particle data.

whereas final harvest pools were evaluated for both genomic titer and capsid titer by ddPCR and ELISA, respectively.

All bioreactor systems exhibited their highest titers in the lysate fraction, representing approximately 60% of the total titer, with 35% in the supernatant fraction and minimal recovery observed in the PBS-wash fraction at approximately 5% of the total titer .

Regarding total viral particles and genomes, although the relative contribution of each fraction varied somewhat between scales, the average genomic titers from final harvest pools remained consistent across scales. Viral genome averages were approximately 1×10^{10} viral genomes/cm² for all scales. A lower viral-particle count was observed in the iCELLis 500+ bioreactor, accompanied by a higher proportion of full capsids. The source of this variability cannot be determined from a single production batch.

To summarize, similarities were observed in harvest function yields, viral particle counts, and full capsid percentages, with some variability. Genomic titers from the harvest pools remained consistent across all scales.

TRANSLATION INSIGHT

This case study demonstrates the scalability of the iCELLis platform and its ability to support a robust and consistent AAV production process. Consistent cell growth, metabolite profiles, and titer outputs were observed across all three scales. The iCELLis bioreactor system contributes to de-risking biomanufacturing processes by providing efficient and reproducible performance across scales. Overall, the implementation of advanced, fully closed manufacturing platforms can accelerate the broader adoption of gene therapies by enabling more efficient, scalable, and cost-effective production.

Q&A

Sara Krekels (left), **Anne MacIntyre** (right)

Q How do you introduce the transfection complex into the vessels?

AM Depending on the size of the transfection complex, we either used a bag or a large shake flask. In both cases, we ensured that the tubing, attached to either the bag or the shake flask, was sufficiently large to facilitate transferring the material from the bag into the vessel as quickly as possible. We then applied light pressure using a hand pump to either the bag or the shake flask and allowed gravity to complete the transfer. In this case study, we did not use any peristaltic pumps or similar equipment to introduce the material.

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Q Was the overnight endonuclease lysis step performed inside the bioreactor or after harvest?

AM For the harvest, we added the lysis buffer into the vessel for the overnight lysis. We removed the supernatant and then added the lysis buffer for the overnight incubation. The solution within the vessel overnight is the lysis buffer, including the endonuclease.

Q Do you see a future for processes that rely on adherent cell cultures?

SK Yes – some people believe the future lies in suspension systems because adherent processes are viewed as less scalable. However, when using a bioreactor like iCELLis, industrial scales can be reached very easily. We are happy to support either approach, based on preference and intended application.

Additionally, in some cases, cells do not perform as well in suspension after being adapted from adherent culture, and the adaptation process itself can be time-consuming. Keeping cells adherent allows production to reach industrial scale, with cells held securely in the fixed bed. Media exchanges or perfusion become straightforward, without the need for tangential flow filtration or other cell-retention devices. Furthermore, harvests from adherent systems are often clearer than those from suspension bioreactors with less host-cell debris.

Q What were your observations around foaming in the iCELLis 50 bioreactor?

AM For anyone who has used the iCELLis platform, specifically the iCELLis 500+ bioreactor, we do observe foaming, especially when using media that contains serum during the process. When we originally operated the iCELLis 50 bioreactor, we kept this in mind because we were not exactly sure what we would see. However, it turned out that we observed very minimal foaming within the iCELLis 50 bioreactor.

BIOGRAPHIES

Sara Krekels is a field application specialist who supports biomanufacturing companies across Europe in their implementation, optimization, and scale-up of single-use bioreactor processes. With 9 years of experience on the iCELLis™ fixed bed bioreactor platform and a strong foundation from previous quality and product engineering roles, she bridges technical insight with practical application to accelerate biomanufacturing success.

Sara Krekels, Senior Upstream Field Application Specialist, Cytiva, Aarschot, Belgium

Anne MacIntyre is a Senior Scientist for Cytiva R&D supporting the advancement and implementation of cutting-edge products and processes for advanced therapy medicinal products. Having accumulated 20 years of industry insight into upstream process development across both adherent and suspension cell culture platforms, Anne now concentrates on gene therapy viral vector applications and technical support, including the iCELLis™ fixed-bed bioreactor. Her extensive experience has enabled her to provide effective solutions to a wide range of bioprocessing challenges.

Anne MacIntyre, Senior Scientist, Viral Vector R&D Upstream, Cytiva, Westborough, MA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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INNOVATOR INSIGHT

Accelerating AAV process development with high-throughput strategies

Kevin Vera, Praveen Kusumanchi, Dan Matuszek, Junyan Zhang, Alejandro Becerra, and Pouria Motevalian

Accelerating process development is essential for advancing viral vector manufacturing and enabling broader access to gene therapies. Advanced approaches, such as high-throughput process and analytical development (HTPAD), can provide a powerful framework to achieve this by combining automated experimentation with integrated analytics. Through parallelized chromatography screening and data-driven optimization, HTPAD compresses downstream development timelines, enhances scalability, and reduces costs. This article showcases case studies demonstrating its effectiveness in refining viral vector purification strategies, improving process robustness, and offering adaptable solutions applicable across multiple AAV serotypes.

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ADVANCING AAV-BASED GENE THERAPIES WITH HIGH-THROUGHPUT PROCESS DEVELOPMENT

Recombinant adeno-associated virus (AAV) remains the predominant delivery modality in the gene therapy field. While lentiviral and herpes simplex virus vectors contribute smaller portions, and non-viral approaches are expanding but still represent a minority, AAVs account for approximately 80% of all viral vectors used in gene therapies. Capsid engineering strategies, including peptide display and shuffling, are being applied to fine-tune tropism and immune profiles, while genome engineering approaches, such as introducing mutations in the inverted terminal repeats and incorporating tissue-specific promoters, aim to enhance expression and safety.

Each innovation can significantly influence how these vectors are incorporated into existing manufacturing platforms. To keep pace, both processes and assays must remain flexible while maintaining efficiency. Advanced strategies such as high-throughput process and analytical development (HTPAD) have been designed precisely for such an environment. This approach enables rapid screening of hundreds of conditions, swift transition to

small-scale comparability studies, and the use of analytics to verify product quality as designs evolve.

OVERVIEW OF THE TRADITIONAL AAV DOWNSTREAM PROCESSING WORKFLOW

In a canonical AAV downstream processing workflow, the process generally begins with a detergent treatment used to lyse producer cells and release the vector. This is followed by filtration to remove larger cellular debris through depth filtration or microfiltration. Many programs incorporate optional tangential flow filtration (TFF) steps for buffer exchange and volume management; however, these are implemented based on specific process design choices [1].

Affinity chromatography is then used to capture the AAV vector and remove many standard impurities, but it is not effective for separating process-related impurities such as empty capsids. This limitation is addressed in the subsequent purification step, typically anion exchange (AEX) chromatography, which exploits differences in the isoelectric points of empty and full capsids. In the final stages of downstream processing, viral reductive filtration is performed as an additional safety measure to further reduce larger adventitious viruses. This is then followed by a final TFF step for formulation, buffer exchange, and product concentration before terminal sterile filtration.

Each of these stages involves critical process decisions, such as the selection of detergent type, contact time, filter grade, depth filter combinations, TFF device chemistries and flow conditions, and chromatography parameters including binding and elution conditions [1]. These variables contribute to the complexity of AAV downstream development, which can often extend beyond twelve months. HTPAD offers the potential to reduce this timeline by parallelizing complex chromatography steps, such as resin or buffer screening, and integrating rapid analytics to enable data-driven learning in days rather than weeks.

STREAMLINING AAV DOWNSTREAM PROCESSING THROUGH HIGH-THROUGHPUT PROCESS ANALYTICAL DEVELOPMENT

Traditional AAV process development typically follows a linear progression, beginning with plasmid, cell line, and viral screening work followed by upstream process development and optimization. This leads in turn to development and then optimization of the downstream process, before proceeding to process verification, scale-up, and manufacturing. Together, the timeline for progressing through these stages frequently extends beyond one year.

HTPAD enables high-throughput strategies to be integrated at two key stages: upstream and downstream process development, both of which are coupled with rapid analytics. In practice, this involves parallel Design of Experiments (DoE) runs in upstream development using automated small-scale bioreactors, while on the downstream processing side, multiple columns or plate-based resin and buffer screens are employed. Streamlined analytics enable fast in-process testing for titer, empty-full capsid ratio, and process-related impurities such as host cell protein and host cell DNA.

These capabilities enable compressed loops whereby experiments can be conducted on day one, analytics completed on day two, insights gathered on day three, and process adjustments implemented on day four. As a result, optimization no longer requires weeks for each iteration. This approach consistently reduces development timelines by several months while

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enhancing process robustness for scale-up and pilot production.

SCALING CHROMATOGRAPHY FROM SCREENING TO PROCESS DEVELOPMENT

To scale chromatography from screening to process development, different HTPAD technologies can be applied. At the smallest scales, resin plates represent the entry point, offering 96-condition throughput for rapid screening. These formats are particularly useful for mapping how different resins interact with the product and for testing variables such as buffer pH, conductivity, and specific buffer additives. However, the binding interactions observed within these plates are static rather than dynamic and are therefore not fully predictive of true column chromatography, which operates under dynamic flow conditions.

At medium scales, RoboColumn™ prepacked columns can be utilized. Although throughput decreases to approximately eight conditions, depending on the liquid handling system used, these tools provide scale-down fidelity. They enable true dynamic binding and more representative pressure, flow, and elution behaviours. Similarly, resin-packed pipette tips offer a related approach, promising many of the same advantages as RoboColumn but without the requirement for costly liquid handling systems.

Finally, findings from small-scale experiments can be validated using conventional columns, such as on a small ÄKTA avant™ 25 system, typically at the 1 mL scale with one to three conditions. This stage is used to lock key parameters and assess overall process robustness.

In essence, this workflow functions as a funnel: beginning broadly and rapidly to eliminate non-viable conditions, then progressively narrowing toward more realistic conditions to reduce risks during scale-up and transfer runs.

CONSIDERATIONS FOR AEX FULL CAPSID ENRICHMENT

When performing more sensitive AAV purification steps, particularly AEX chromatography, it is crucial to consider several factors to help ensure reliable enrichment of viral capsids.

AAV capsids are generally about 25 nm in diameter, allowing them to diffuse into large-pore adsorbents. As a result, pore architecture and flow conditions play a critical role in determining mass transfer efficiency and binding behaviour.

Most AAV-based products are rarely composed of a single homogeneous species. Instead, they typically consist of a complex mixture that includes empty capsids, partial capsids, full capsids, and in some cases, aggregates. Additionally, the charge characteristics of these components are highly nuanced – predicted isoelectric points often differ significantly from experimentally determined values. This discrepancy is likely caused by factors such as post-translational modifications that alter the apparent surface charge of the capsids.

This variability manifests as heterogeneity in AEX chromatograms. Multiple peaks may appear where a single peak would be expected, or the elution order of species may shift. For example, a typical AAV9 full capsid peak often elutes earlier than the corresponding empty capsid peak during a gradient elution.

CRITICAL PARAMETERS & ANALYTICAL STRATEGIES FOR OPTIMIZING AEX CHROMATOGRAPHY IN AAV PURIFICATION

Several critical parameters must be assessed when applying AEX chromatography to AAV purification. Typical operating pH values range from 8.5 and 9.5, with Bis-Tris propane frequently serving as the buffer system of choice due to its pKa being

well aligned with the desired operating range. Salt selection is another influential factor: certain divalent ions, particularly magnesium, can significantly affect separation behaviour by modulating charge interactions and altering elution profiles [2].

Elution strategies commonly include linear gradients, isocratic steps, or hybrid approaches that combine both. When optimizing salt combinations, the use of multiple salt species (dual or even triple salt systems) can help fine-tune selectivity. Fractionation and pooling strategies are also crucial – broad collection windows may cap the achievable percentage of full capsids, while overly narrow windows can limit total recovery, particularly when the separation strength is relatively weak. Although not widely practiced, some groups have begun implementing iterative AEX processing, in which the same material is run through multiple AEX columns to maximize purity. While this can improve empty-full capsid separation, it introduces considerable additional complexity, increases material consumption, and extends processing time, which restricts its practicality.

These process decisions must be firmly supported by analytical data. Key analytical methods include PCR and ELISA for capsid and genome quantification, mass photometry for rapid assessment of empty-full ratios, analytical AEX for monitoring peak behaviour, transmission electron microscopy for structural evaluation, and analytical ultracentrifugation for definitive empty, full, and partially filled species separations.

CASE STUDY: ESTABLISHING & VALIDATING AN AUTOMATED HIGH-THROUGHPUT AEX WORKFLOW FOR AAV PURIFICATION

Advanced platforms such as RoboColumn enable ultra-scale-down AEX experiments to be performed in parallel, integrating several screening strategies together with the analytical methods required to verify empty-full capsid enrichment. This approach serves as the crucial bridge between rapid small-scale screening and confident scale-up, ensuring that process parameters identified through high-throughput experimentation translate effectively to larger production scales.

In a proprietary study, implementation of HTPAD began with the verification of the system's ability to generate a pseudo-linear gradient method using a liquid handling platform. Liquid handling scripts were then developed and tested, followed by confirmation of AEX method reproducibility. A short experimental design was executed on RoboColumn™, screening several salt combinations. Results from these initial experiments were verified on 1 mL columns and subsequently confirmed across an additional AAV serotype.

The resin selected for this work was POROS™ 50 HQ, an AEX resin with a mixed amine surface containing approximately 60% quaternized polyethylenimine. The resin possesses a pore size of roughly 200 nm, which is sufficiently large to permit AAV diffusion into the beads. The beads themselves are rigid and 50 µm in size, supporting high-resolution separation and fast flow rates [3].

In summary, a robust resin with an extensive performance record in bioproduction manufacturing was selected, a reproducible automated method was established, and the small-scale process was successfully confirmed at a 1 mL scale before being extended to broader serotype testing.

Buffer gradient method verification

Establishing the pseudo-linear gradient method served primarily to confirm that the gradient mechanics functioned as intended and could perform on the liquid handling system reproducibly. Using a Tecan Fluent® 1080 platform and 2 mL deep-well plates, a

sodium chloride elution buffer series was prepared, producing a near-linear gradient conductivity ramp across several column volumes (CVs), as illustrated in Figure 1.

Two practical considerations guided this setup. Firstly, higher working volumes were selected to remain within the liquid handler's lower-volume limits. Secondly, the prepared gradient was verified by measuring conductivity readings across each generated CV to ensure that the gradient followed a linear trend. This verification step was essential to support consistency and comparability across RoboColumn runs.

With the gradient confirmed, parallel AEX experiments could be conducted with confidence. Consistent gradient performance allowed for reproducible chromatographic profiles, showing the same curve shapes and micro-steps, ensuring that any observed differences between runs could be attributed to process variables, such as pH, salt type, and buffer composition, rather than uncontrolled gradient variability.

AEX liquid handling script creation and execution

With confidence established in the pseudo-linear gradient method, the approach was applied to an AAV5-based product to demonstrate the method's full capability. Using the liquid handling script, a 60-CV gradient was executed, with each step collected as a single-CV fraction. Afterwards, UV absorbance at 260 nm and 280 nm was measured for each fraction.

The resulting overlay revealed distinct and interpretable peak profiles (Figure 2A). As the process moved through the apex of the peak, an increase in the percentage of full capsids was observed, confirming effective separation.

Further resolution is illustrated in Figure 2B, where two specific CVs – 35 and 36 – show a shift toward a higher proportion of full capsids as the peak apex was approached.

In essence, collecting single-CV fractions provided sufficient resolution to define a suitable pooling window that maximized the proportion of full capsids while minimizing empty or partially filled carryover. By analyzing UV absorbance ratios alone, rapid and reliable pooling decisions could be made, reducing the need for small-scale reruns and improving overall process efficiency.

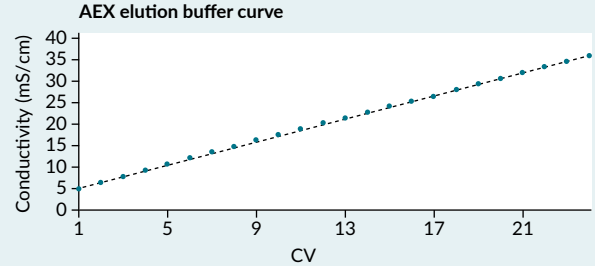
Evaluating HTPAD method reproducibility

To evaluate reproducibility of the HTPAD method, the same AEX script was run across seven arms of the Tecan Fluent 1080 system. As shown in Figure 3, the overlays demonstrated close agreement in the 260/280 absorbance ratio and consistency in the ratio between the two across each CV. The peak position and shape were consistently reproduced across all seven runs.

A standardized pooling window was then applied to each run and the outcomes were measured. Full capsid composition was assessed by mass photometry, averaging approximately 73 ± 2%, while vector recovery, measured by droplet digital (dd)PCR, averaged around 64 ± 3%. These

FIGURE 1

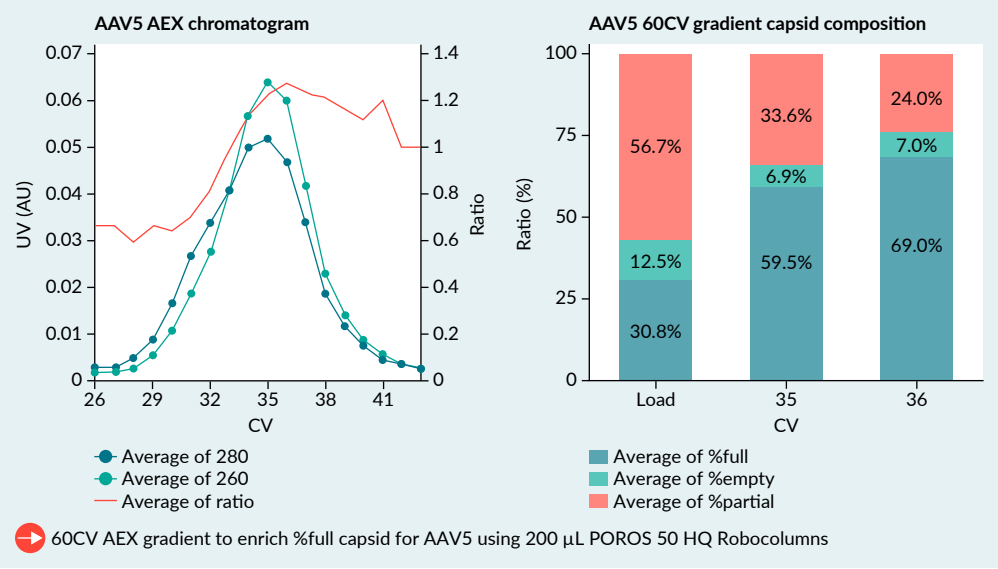
Verification of the pseudo-linear gradient method on the liquid handling platform.



- Elution buffer gradient created with NaCL in a 2 ml deep well plate using Tecan 1080 Fluent system
- Higher volumes are necessary to support lower limit of liquid handler

FIGURE 2

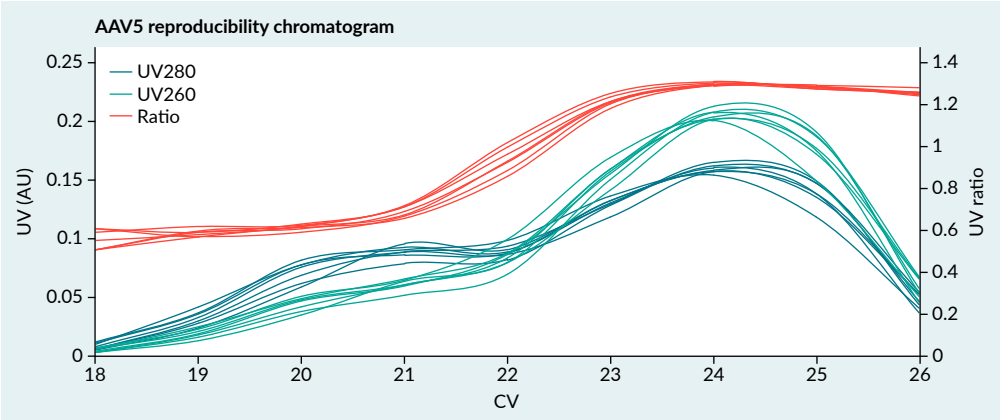
AAV5 AEX chromatogram and AAV5 60CV gradient capsid composition.



(A) Overlay of UV absorbance at 280 nm (teal) and 260 nm (turquoise), with the 260/280 ratio (red) indicating the elution region of genome-containing (full) capsids along the gradient. (B) Bar chart showing capsid composition for the load and two elution fractions (CVs 35 and 36), demonstrating an increase in the percentage of full capsids at the peak apex.

FIGURE 3

Reproducibility of the automated HTPAD AEX method across multiple arms of the Tecan Fluent 1080 system.



Average full capsid ratio via MP (%)	73 ± 2
Average Vg recovery via ddPCR (%)	64 ± 3

findings indicated two key points: firstly, the automated gradient preparation and fractionation steps produced consistent chromatograms; secondly, the pooling rule translated effectively into stable product outcomes. With reproducibility confirmed, the process could be expanded to broader experimental designs without concern for

run-to-run variability that could obscure or mask true effects.

AEX method experimental design and execution

For the exploratory setup, all conditions were run using the POROS 50 HQ RoboColumn with a fixed pH of 9.0. Two parameters were varied: the load dilution ionic strength (10-16 mM) and the elution buffer salt architecture (single, dual, and triple-salt schemes at different ionic strengths). In several runs, low concentrations of salts were maintained consistently in both the load and dilution solutions to identify any potential effects these factors might have. The overall intent of the experimental design was to assess how ion composition and ionic strength influenced analyte selectivity and peak behavior.

Based on the results of seven parallel runs, the separation windows and apex CVs shifted and varied according to the elution buffer composition - as expected, since this variation was a key focus of the investigation. A simple and consistent pooling rule was applied, selecting the top three fractions based on UV ratio and magnitude to determine which samples would proceed to further analysis.

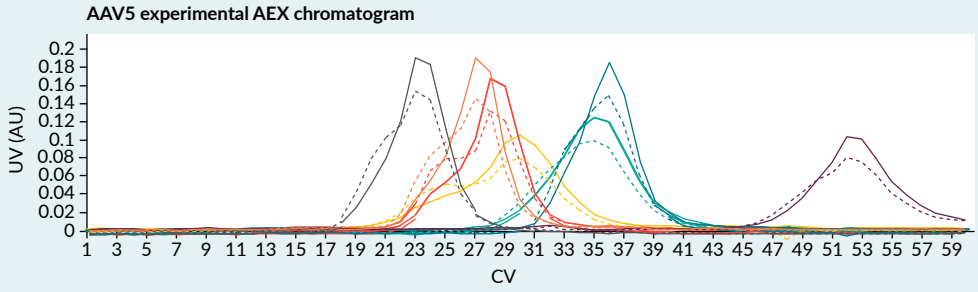
Among the tested conditions, numbers 3, 6, and 7 showed the highest percentage of full capsids, ranging from 78–85% (Figure 4).

To assess whether any salts had an impact on AAV stability, dynamic light scattering (DLS) measurements were also conducted. All vectors exhibited a hydrodynamic radius of approximately 14.4 ± 0.2 nm, with monodisperse peaks, confirming that none of the salts adversely affected expected size of the AAV capsids.

Overall, the method effectively discriminated among salt types. The A260/A280 overlays successfully identified the full capsid enrichment windows, and the use of parallelization enabled suitable condition selection within a single experimental

FIGURE 4

Parallel AEX runs showing UV absorbance profiles and capsid enrichment outcomes.



Condition no.	Affinity eluate	1	2	3	4	5	6	7
Yield (%)	–	51	32	48	56	47	50	54
Full (%)	31	67	78	79	70	74	78	85

- 7 conditions run in parallel
- DLS confirmed 14.4 ± 0.2 nm radius, with PDI indicating monodispersity

*Mock pool of top 3 fractions based on UV ratio and UV magnitude

Each condition represents paired UV traces, with solid lines indicating absorbance at 260 nm (A260) and dashed lines at 280 nm (A280); matching colors correspond to the same run. The accompanying summary table displays yield and percentage of full capsids for all conditions, illustrating variability in separation behavior and enrichment efficiency across different elution buffer compositions.

set, achieving approximately a seven-fold compression compared with individual experimentation.

1 mL column verification

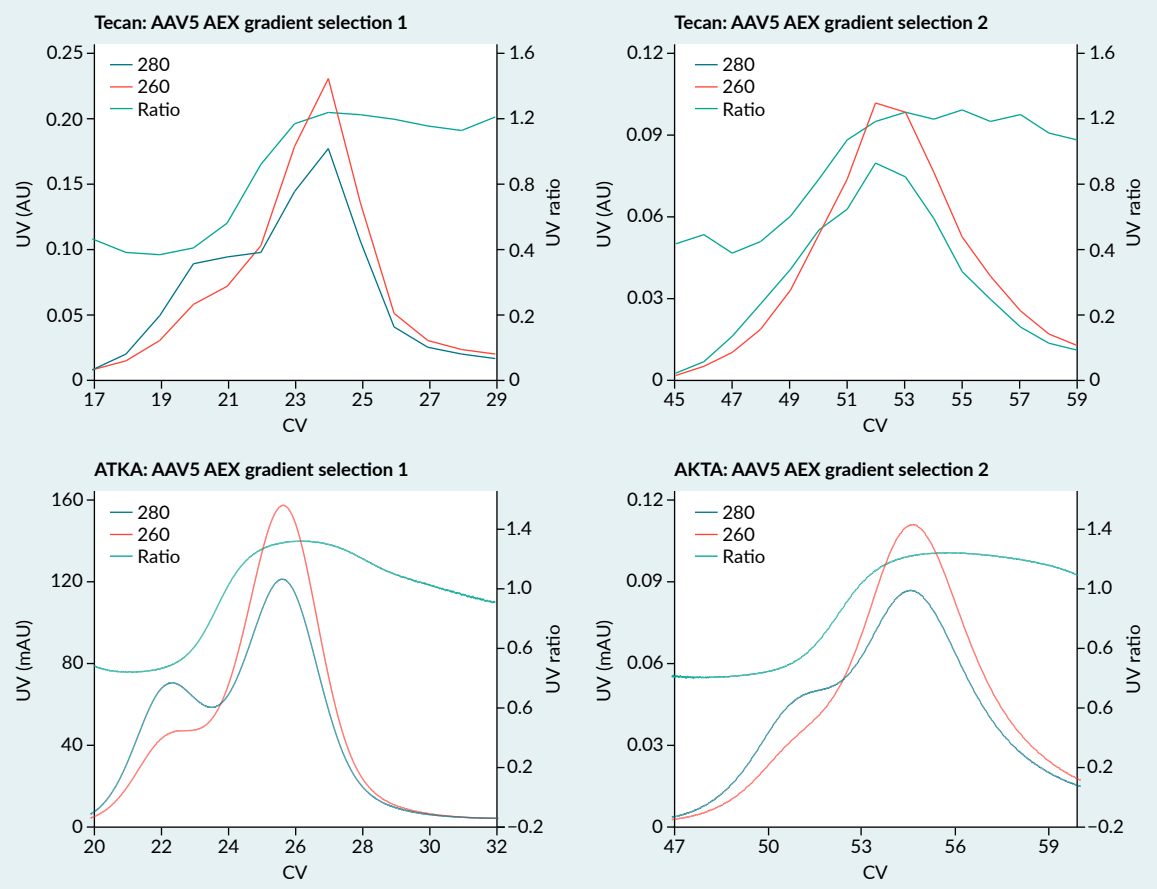
For the verification runs, two AEX gradients were selected from the RoboColumn screening and verified on 1 mL columns using an ÄKTA avant™ 25 chromatography system. As shown in Figure 5, A260/A280 peak shapes, apex CVs, and UV ratio trends aligned consistently across both Tecan and ÄKTA platforms. Although the ÄKTA data were more discretized, resulting in a greater number of data points and smoother curves, the pooling window defined by the A260/A280 ratio matched that observed in the RoboColumn runs on the Tecan system.

These findings confirmed that the dynamic behavior scaled appropriately between formats. The same gradient profiles, similar peak shapes, and consistent fraction boundaries were maintained. In practical terms, this outcome reduces the risk associated with process transfers. The parameters for linear gradient elutions, once established at the ultra–scale-down level, were shown to remain robust when applied to conventional column formats.

As a demonstration, hypothetical pools from various elution fractions were plotted

FIGURE 5

Comparison of AEX gradient performance between Tecan and ÄKTA chromatogramphy systems.



The top panels show UV absorbance profiles from RoboColumn runs on the Tecan system, while the bottom panels display corresponding 1 mL ÄKTA verification runs. Consistent A260/A280 peak shapes, apex positions, and pooling windows confirm that gradient behavior and separation performance scale reliably between automated small-scale and bench-scale chromatography formats.

to illustrate how yield and purity change in relation to one another. As shown in Figure 6, the curves corresponding to the two gradient selections aligned closely between the Tecan and ÄKTA systems, showing negligible differences. A clear trade-off emerged: expanding the fraction pool increased yield, but purity (or the percentage full capsids) began to decline. Conversely, tightening the pooling conditions improved purity while reducing yield.

These trends highlight the decision-making flexibility within process development. When a program prioritizes potency and dose efficiency, selecting a higher purity window is advantageous. However, when manufacturing throughput or dose availability presents a constraint, accepting a slightly broader pool with marginally lower purity becomes a practical choice.

Ultimately, the advantage of HTPAD lies in its ability to generate these performance curves quickly and to refine them as assays or product targets evolve.

Multi-serotype method confirmation

Lastly, to assess the applicability of the HTPAD method across different serotypes, an internally produced AAV9 product was tested. A well-established AEX enrichment process, originally developed at a 5 mL scale, was scaled down to the RoboColumn format. The UV 260/280 profiles aligned closely between the two scales, exhibiting consistent peak positions, shapes, and ratios (Figure 7A). This confirmed that both the dynamic behaviour and pooling windows translated effectively to the smaller scale.

Furthermore, the capsid composition plot across all CVs displayed an expected trend – the percentage full capsids increased through the apex fractions, while partial and empty capsids decreased (Figure 7B).

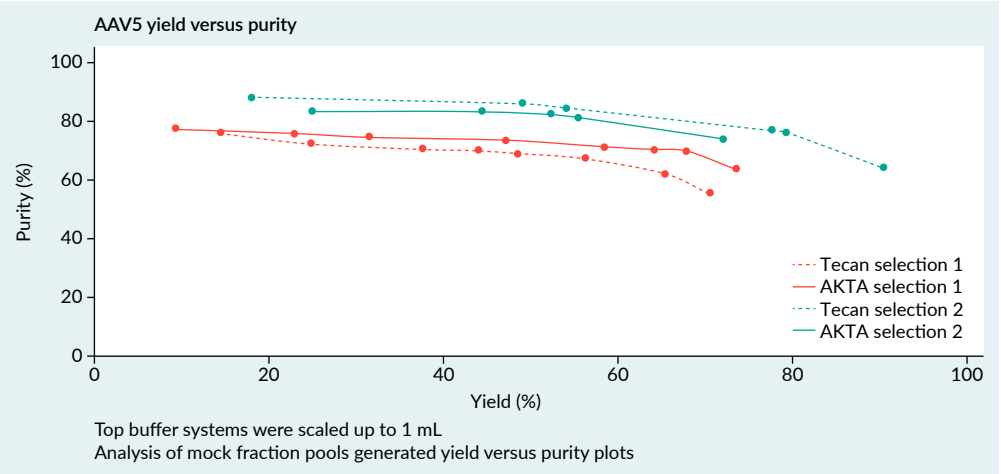
In summary, the same method architecture used for AAV5 performed equivalently for AAV9, confirming that the HTPAD workflow is applicable across different AAV serotypes.

Key benefits of HTPAD in AAV process development

In conclusion, there are three key advantages of using HTPAD-based AAV process development: speed, cost-efficiency, and scalability.

FIGURE 6

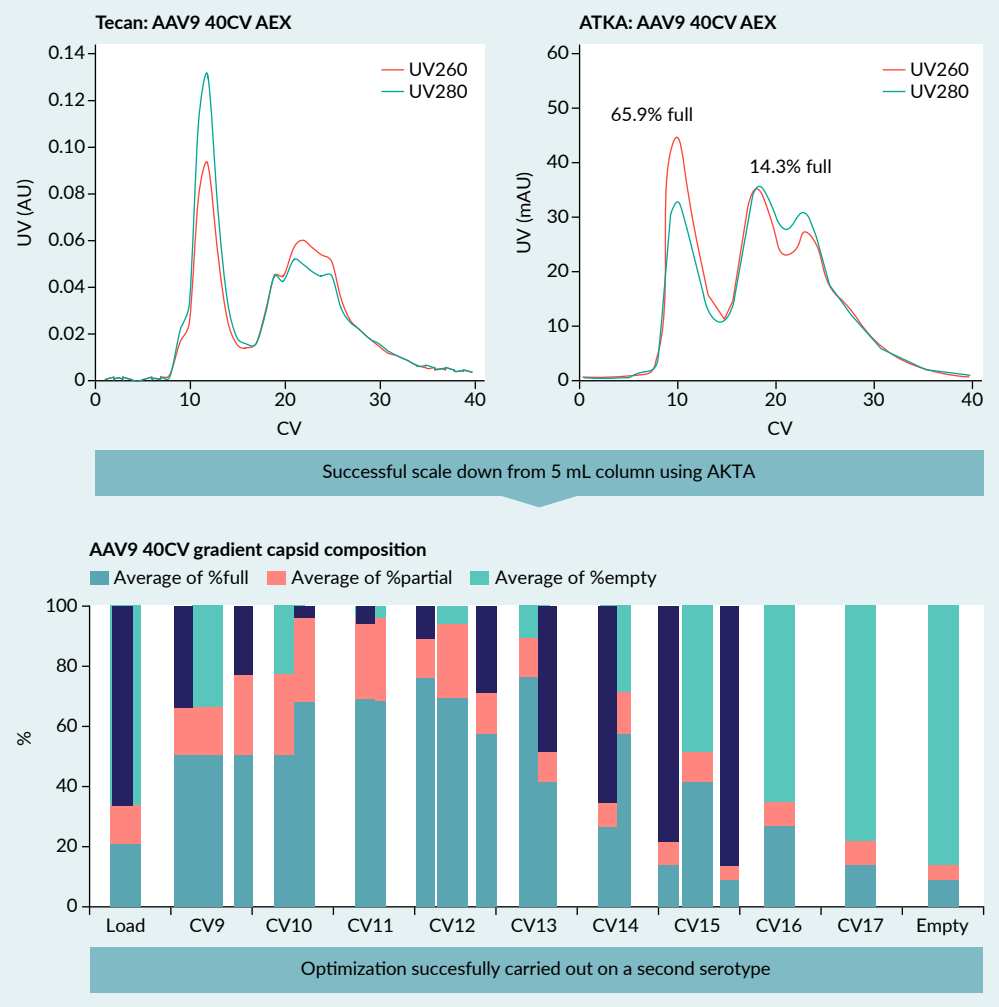
Relationship between yield and purity across pooling windows for AEX separations.



Top buffer systems were scaled up to 1 mL
Analysis of mock fraction pools generated yield versus purity plots

FIGURE 7

Cross-serotype validation of the HTPAD AEX workflow.



(A) UV 260/280 profiles for AAV9 show consistent peak alignment between 5 mL and RoboColumn scales. (B) Capsid composition across CVs indicates increased full capsid enrichment at the peak apex, confirming scalability and applicability across serotypes.

- The AEX development work was completed within a week, showing that combining RoboColumn technology and automated chromatography enabled roughly an eight-fold acceleration compared with single-run workflows;
- The experimental footprint was substantially reduced, achieving more than an 85% cost reduction. This improvement was driven by a five-fold decrease in the use of consumable use, product, buffer, and full-time equivalent hours per study;
- The developed methods transferred seamlessly to bench-scale chromatography, as demonstrated by the 1 mL ÄKTA avant verification runs, demonstrating scalability.

In practical terms, these outcomes translated into faster cycles for condition optimization, clearer pooling rules guided by orthogonal analytics, and reduced risk during process scale-up. Overall, the connected process development and analytical development workflow enabled the implementation of high-throughput experimentation with

robust analytics, transforming downstream process development into a data-driven and repeatable practice.

SUMMARY

Advanced high-throughput strategies can accelerate AAV downstream process development by enabling rapid, parallel chromatography screening and robust analytical validation. Through automated HTPAD tools such as liquid handlers, process parameters can be optimized efficiently, ensuring reproducibility and scalability across different serotypes. Additionally, by integrating data-driven analytics and small-scale experimentation, HTPAD reduces development timelines, lowers material and labor costs, and improves process robustness. This approach transforms traditional AAV downstream processing into a faster, more efficient and adaptable workflow, supporting the growing demands of gene therapy production.

Q&A

Dan Matuszek

Q How does the HTPAD approach compare to typical AAV chromatography development timelines with respect to speed and resource use?

DM The HTPAD approach offers a substantial advantage in both speed and resource efficiency compared with traditional AAV chromatography development. Unless multiple FPLC systems are available in the laboratory, the number of runs that can be performed in parallel is inherently limited. However, by using the Tecan liquid handling script with HTPAD methods, eight runs can currently be performed simultaneously, compared with only one or two runs when using two FPLC systems.

Importantly, the benefit is not limited to schedule compression. HTPAD also reduces the amount of raw materials required - including product, buffers, consumables, and operator time – making each experimental cycle more cost-efficient while accelerating overall development.

Q How do you handle aggregate detection and mitigation during AEX?

DM Aggregate detection and mitigation during AEX chromatography has become an increasing concern. Recent data have shown changes in DLS measurements – specifically, shifts in hydrodynamic radius – that vary depending on the

elution salts used. Although the underlying causes are not yet fully understood, and no significant decreases have been observed in other assays such as infectivity, it remains an important factor to monitor as a part of process evaluation.

Mitigation steps involve returning to the initial AEX step if parameters begin to drift out of specification. This includes reassessing load dilution conditions, the choice and concentration of elution salts, and determining the most suitable conditions for the specific molecule. It is important to evaluate whether the pursuit of higher purity using a particular salt may be compromising viral stability or infectivity, or whether prioritizing recovery while maintaining acceptable stability is more appropriate. Ultimately, it all boils down to deliberate design choices that balance purity, recovery, and product integrity.

Q Do you find that high empty-full ratio samples purify better than low empty-full samples? Do you see the ratio affecting downstream processing in general?

DM Regarding whether a high empty-full capsid ratio is observed purifying better or worse, the answer is generally no; the outcome depends heavily on how well the process has been developed. Typically, in cases where the AEX process is not highly developed – such as in early research or expedited clinical programs – AEX typically yields about a 1.5-fold enrichment. Once the process is fully developed, significantly higher enrichment levels are achievable.

Starting with a higher percentage of full capsids is generally advantageous. Many aspects can be optimized during the upstream phase before progressing to downstream processing to improve this percentage. It is strongly recommended to optimize upstream parameters first, as this can significantly reduce the burden on AEX to generate purity solely through separation.

During downstream processing, a lower starting percentage of full capsids could lead to narrower separation windows and lower recovery at high-purity targets. Conversely, material with higher initial full capsids could support broader separation windows and higher recovery at the same purity specification.

REFERENCES

1. Park MT, Verma A, Froelich CA, *et al.* Process and quality considerations for recombinant adeno-associated virus manufacturing platforms. *Trends Biotechnol.* 2025; 43, 1921–1937.
2. Lavoie RA, Zugates JT, Cheeseman AT, *et al.* Enrichment of adeno-associated virus serotype 5 full capsids by anion exchange chromatography with dual salt elution gradients. *Biotechnol. Bioeng.* 2023; 120, 2953–2968.
3. Thermo Scientific. *POROS Anion Exchange Resins: XQ, 50 HQ, 50 PI, and 50 D* (Rev.C). 2020. https://documents.thermofisher.com/TFS-Assets/LSG/manuals/100031320_POROS_AnionExchResins_PI.pdf.

AFFILIATIONS

Kevin Vera, Praveen Kusumanchi, Dan Matuszek, Junyan Zhang, Alejandro Becerra, and Pouria Motevalian, Thermo Fisher Scientific

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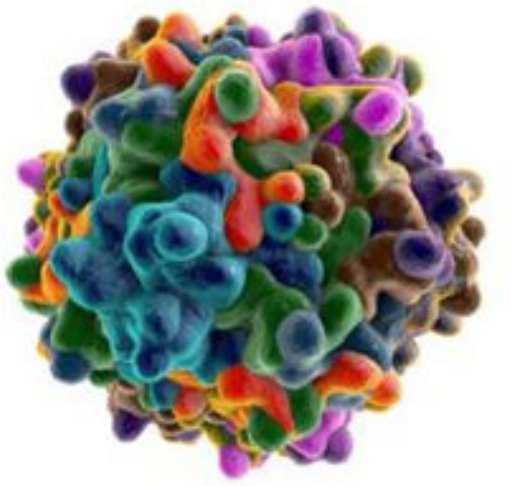

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INNOVATOR INSIGHT

Streamlined dPCR workflow for lentiviral characterization and QC of cell and gene therapies

Ruth Klaver, Miroslav Vranes, and David Dobnik

Lentiviral vectors form the basis of many *ex vivo* gene-modified cell therapies, including CAR-T and other engineered T cell products. As manufacturing grows, analytical tools must keep up with changing demands for safety, potency, and consistency. Digital PCR has become a reliable, absolute method for measuring key quality attributes of lentiviral products, such as vector genome titer, integrated vector copy number, and the absence of replication-competent lentivirus. This article outlines a streamlined digital PCR workflow that uses CGT-specific assays and a direct lysis protocol to enhance efficiency and reproducibility while lowering operator burden. Application datasets demonstrate the performance of this integrated workflow across various lentiviral matrices. Collectively, these approaches emphasize the increasing importance of digital PCR in supporting QC strategies aligned with regulatory expectations in CGT manufacturing.

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Lentiviral vectors (LVVs) are widely used in both clinical and commercial *ex vivo* gene-modified cell therapies, providing stable genome integration, broad tropism, and the ability to deliver complex transgenes. However, achieving consistent product quality relies on precise measurement of critical quality attributes (CQAs), such as physical vector genome titer, integrated vector copy number (VCN), and confirmation of the absence of replication-competent lentivirus (RCL).

Traditional analytical approaches, such as qPCR, ELISA, and cell-based infectivity assays, can be labor-intensive, time-consuming, or prone to variability. Digital PCR (dPCR) provides absolute quantification without the need for external standard curves, enabling improved precision, linearity across broad dynamic ranges, and greater consistency across operators and sites.

The recent expansion of the QIAGEN CGT assay portfolio has equipped users with wet-lab tested assays targeting LVV replication genes, packaging sequences, and common reporter elements, enabling standardized measurement of titer, VCN, and RCL detection.

DETECTION OF REPLICATION-COMPETENT LENTIVIRUS ABSENCE

RCLs can emerge through recombination events during production that lead to restoration of elements required for viral propagation. Detecting these events is a critical safety requirement in the manufacturing of *ex vivo* cell therapy products. dPCR enables sensitive detection of RCL through absolute quantification of VSV-G sequences within genomic DNA.

The QIAcuity™ RCL Quant Kit allowed for the direct evaluation of assay performance with a positive control diluted over a range of 0.35–4000 copies/µl of reaction. Each concentration was tested in the presence of 0, 1, or 10 µg of background genomic DNA to assess potential interference. Across all background DNA levels, positive and negative partitions remained clearly resolved, and measured concentrations were consistent, as shown in Figure 1. These results show that the VSV-G assay maintains its quantification accuracy when using the QIAcuity RCL Quant Kit even in high-background matrices typical of cell therapy workflows.

QUANTIFICATION OF INTEGRATED VECTOR COPY NUMBER

VCN represents the average number of integrated lentiviral genomes per cell and is essential for evaluating genomic safety and product performance. VCN determination relies on quantifying a lentiviral target sequence relative to a genomic DNA (gDNA) reference locus using duplex dPCR and was performed using the QIAcuity™ CGT dPCR Assays.

Lentiviral targets suitable for this analysis include transgene-associated elements, such as GFP or PuroR; regulatory sequences, such as WPRE or CMVp; and integration plasmid elements, including Psi, RRE, and 5′ long terminal repeat (LTR). These assays can be combined with reference targets such as Albumin, RPP30, or RPL32 to calculate the vector copies per cell. The formula for diploid genomes used was:

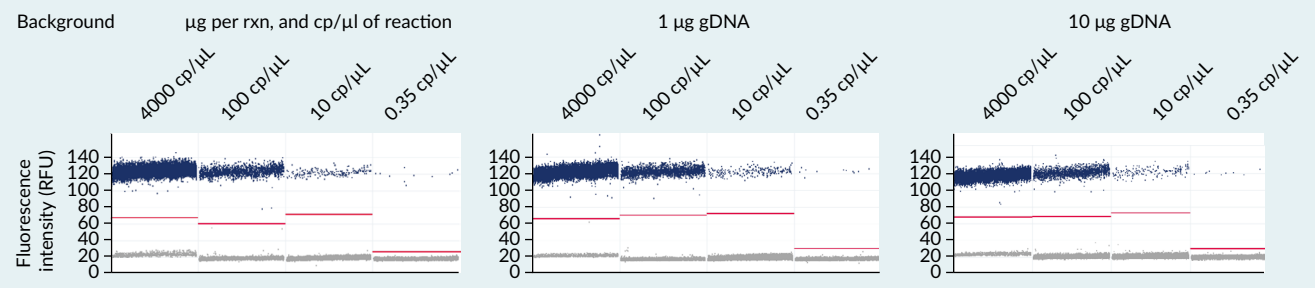
$$\text{VCN} = 2x \frac{\text{vector target copies}}{\text{human reference target copies}}$$

A schematic representation in Figure 2 illustrates the relationship between measured target-to-reference ratios and theoretical VCN values in diploid genomes. A single integrated copy corresponds to one lentiviral target per two reference gene copies, while a VCN of three corresponds to three lentiviral copies per two reference copies.

Assay linearity was evaluated for the RRE target labeled in HEX, and the RPL32 reference labeled in FAM using serial dilutions spanning 0.25–7500 copies/µl of

FIGURE 1

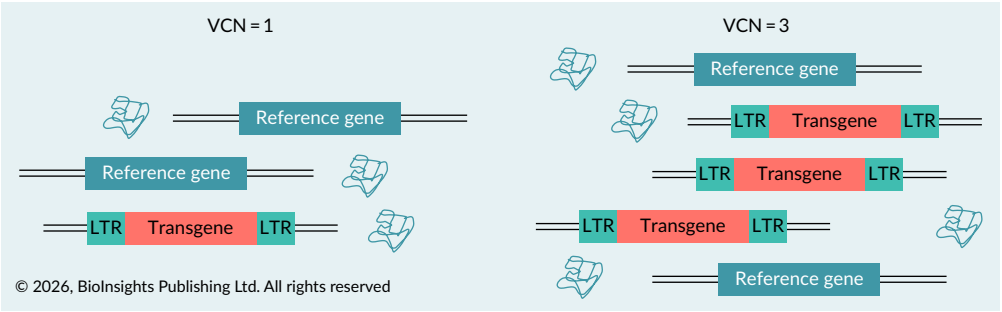
dPCR detection of VSV-G across increasing levels of background DNA.



*cp/µl of reaction. dPCR: digital polymerase chain reaction. VSV-G: vesicular stomatitis virus G.

FIGURE 2

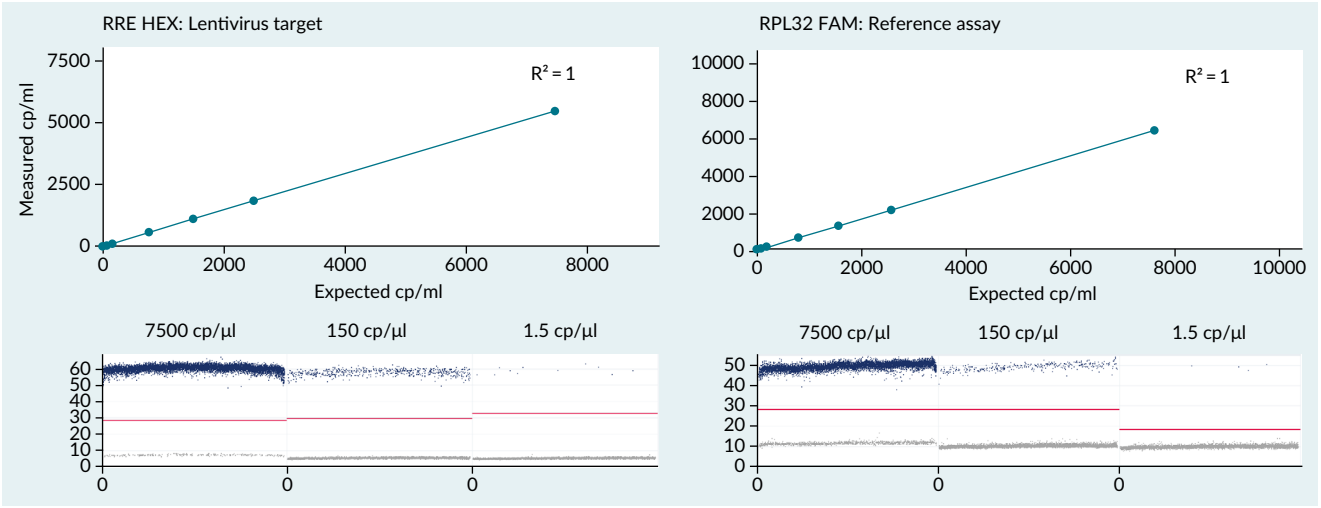
Schematic representation of VCN calculation, where VCN values correspond to the ratio of lentiviral target copies to reference gene copies, with examples for VCN 1 and VCN 3.



 VCN: vector copy number.

FIGURE 3

Linearity of RRE and RPL32 assays across a broad dynamic range with serial dilutions from 0.25–7,500 copies/µl produced proportional increases in partition counts for RRE (HEX) and RPL32 (FAM).



RRE: rev response element.

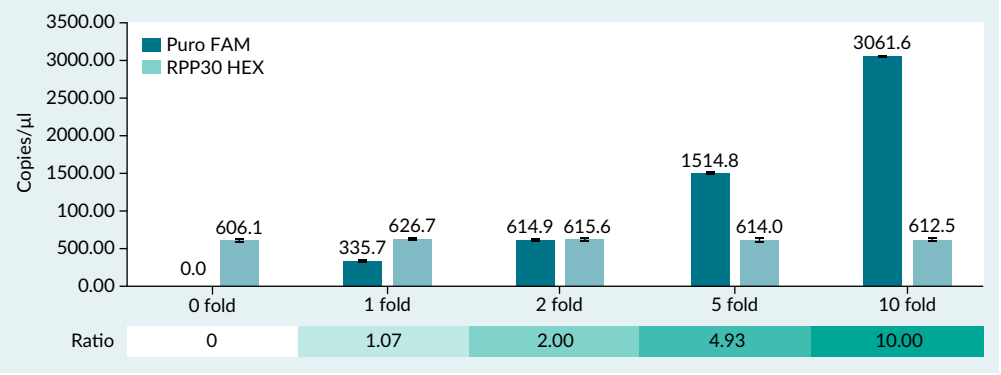
reaction on 8.5k nanoplates. Both assays demonstrated highly linear responses across this range on the QIAcuity dPCR system, supporting their suitability for quantitative applications requiring a broad dynamic range, as shown in Figure 3.

VCN accuracy was further assessed using spike-in experiments simulating VCN values of 0, 1, 2, 5, and 10. Duplex reactions combining the PuroR lentiviral target in FAM with the RPP30 reference gene in HEX produced measured ratios that deviated by <5% from expected values across the simulated VCN range, as shown in Figure 4.

VCN was also quantified in a control cell line with an established VCN of 1, using a duplex pairing of WPRE labeled in HEX with albumin labeled in FAM. The measured value closely matched the expected VCN.

FIGURE 4

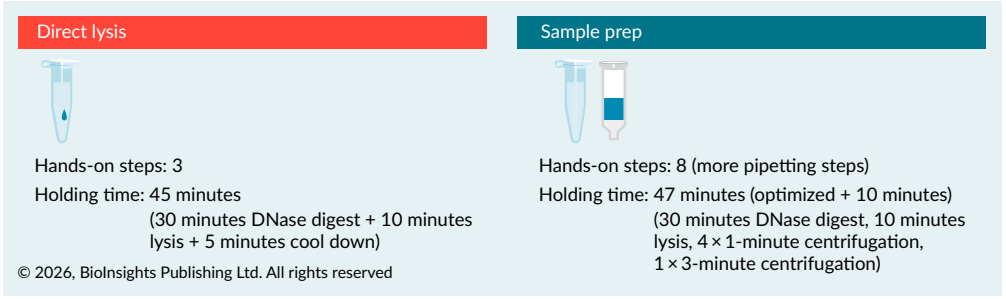
VCN accuracy using PuroR and RPP30, where simulated VCN values from 0–10 showed <5% deviation between expected and measured ratios.



VCN: vector copy number.

FIGURE 5

Comparison of direct lysis and silica membrane-based sample preparation: direct lysis requires three hands-on steps compared with eight for column-based extraction and eliminates multiple centrifugation steps while maintaining a similar overall incubation period.



LENTIVIRAL GENOME TITER WORKFLOW

Accurate determination of lentiviral genome titer is essential for assessing vector production consistency and establishing dosing parameters for downstream cell modification. dPCR can quantify genome-containing particles by targeting lentiviral RNA sequences following the release of viral genomes from capsids. Genome-containing particles were quantified using one-step reverse-transcription dPCR (RT-dPCR) with the QIAcuity dPCR system, QIAcuity CGT dPCR Assays targeting lentiviral sequences, and the QIAcuity OneStep Advanced Probe Kit. Viral RNA was prepared using the CGT Lentivirus Lysis Kit, which combines enzymatic digestion and thermal lysis to release viral genomes directly into solution.

The direct-lysis method incorporates an initial DNase digestion to remove contaminating DNA, followed by Proteinase K treatment. This approach avoids the multiple centrifugation and binding steps required for silica-membrane RNA extraction. A comparative overview of the two methods shown in Figure 5 indicates

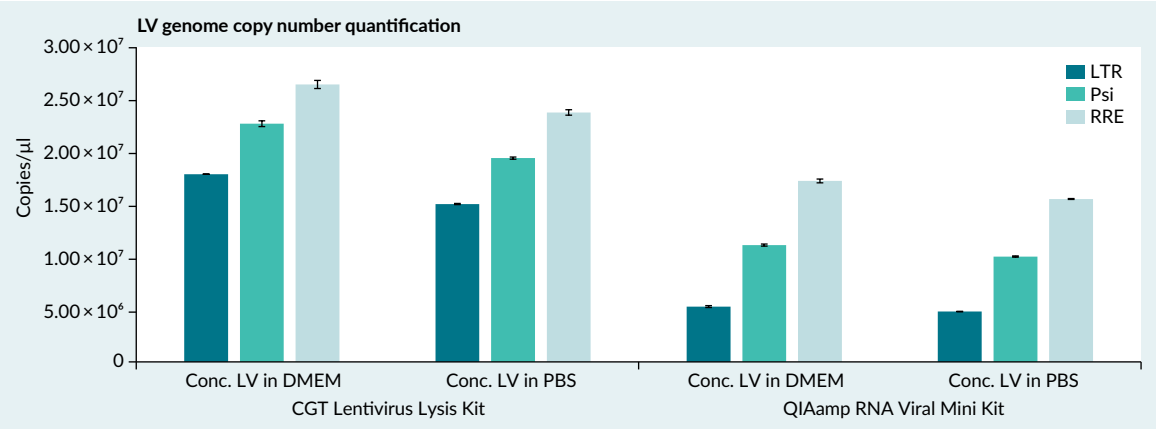
that direct lysis reduces hands-on pipetting steps from 8 to 3, while maintaining the total incubation time required for nuclease digestion and lysis.

The direct lysis workflow was evaluated using concentrated lentiviral samples in different buffer compositions. Across targets including LTR, Psi, and RRE, the direct-lysis method produced higher measured genome copy numbers than column-based extraction in both DMEM and PBS, as shown in Figure 6. This indicated more efficient genome release or improved preservation of template material under the simplified processing conditions.

A similar comparison was performed using lentiviral material from process supernatants, with RNA prepared using the CGT Lentivirus Lysis Kit. In these samples, direct lysis again produced higher genome

FIGURE 6

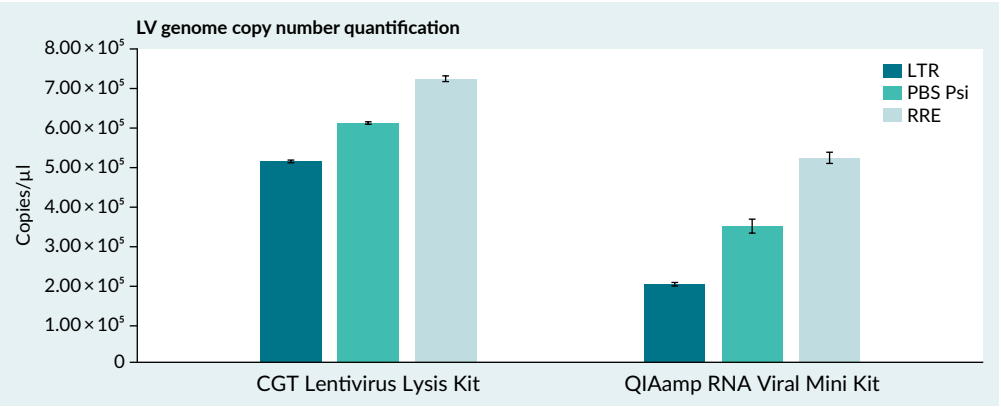
Genome copy number comparison for concentrated lentiviral samples, where direct lysis produced higher measured genome copy numbers than column-based extraction for concentrated samples in both DMEM and PBS across multiple lentiviral targets.



LTR: long terminal repeat. RRE: rev response element. LV: lentiviral.

FIGURE 7

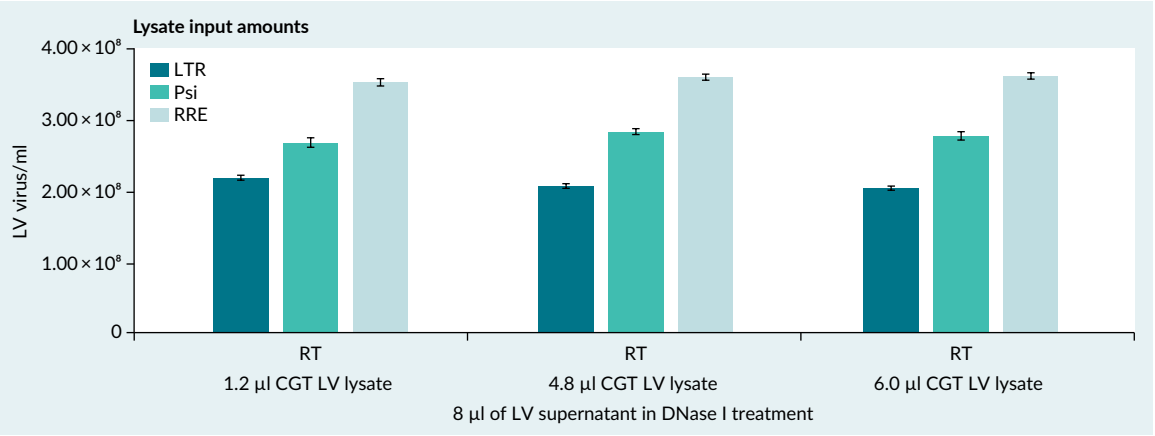
Genome copy number comparison for supernatant samples, where direct lysis generated higher lentiviral genome titers than column-based extraction for supernatant samples across all tested targets.



LTR: long terminal repeat. RRE: rev response element. LV: lentiviral.

FIGURE 8

Tolerance of varying lysate input volumes, where measured lentiviral titers remained stable across lysate input volumes of 1.2 µl , 4.8 µl , and 6.0 µl for LTR, Psi, and RRE targets.



LTR: long terminal repeat. RRE: rev response element.

TABLE 1

Comparison of one- and two-step lentiviral genome quantification.

Samples	LV titer (two-step) (vg/ml)	LV titer (one-step) (vg/ml)	% recovery in one- vs two-step
A	9.22×10^{10}	1.07×10^{11}	116
B	7.09×10^{9}	9.88×10^{9}	139
C	4.27×10^{9}	5.65×10^{9}	132
D	5.96×10^{10}	7.37×10^{10}	124
E	3.26×10^{9}	5.56×10^{9}	171
F	9.38×10^{8}	8.42×10^{8}	90

LV: lentiviral.

copy numbers than the RNA extraction workflow, shown in Figure 7. The results support the suitability of the direct lysis method for in-process sample types that can otherwise be susceptible to variable extraction efficiencies.

The workflow's robustness was evaluated by varying the lysate input volume during the DNase digestion step. Measured titers remained consistent across 1.2, 4.8, and 6.0 µl of lysate input, indicating tolerance to different input volumes and supporting applicability across upstream materials with varying viral loads, as shown in Figure 8.

INDEPENDENT EVALUATION OF THE DIRECT-LYSIS WORKFLOW

An independent assessment at Niba Labs compared QIAcuity-based one-step and two-step RT-dPCR workflows and then evaluated the CGT Lentivirus Lysis Kit against column-based RNA extraction.

The first comparison evaluated genome titers obtained using the two-step workflow against titers obtained with one-step RT-dPCR across six samples, A–F. For the most part, one-step RT-dPCR produced higher measured titers than the two-step

TABLE 2

Comparison of column-based RNA extraction and direct lysis workflows for lentiviral genome quantification.

Samples	LV titer (RNA extraction) (vg/ml)	LV titer (LV lysis) (vg/ml)	% recovery in lysis vs extraction
G	1.07×10^{11}	6.35×10^{10}	59
H	9.88×10^{9}	1.26×10^{10}	128
I	5.65×10^{9}	6.09×10^{9}	108
J	7.37×10^{10}	6.00×10^{10}	81
K	5.56×10^{9}	7.54×10^{9}	136
L	8.42×10^{8}	2.94×10^{9}	349

LV: lentiviral.

TABLE 3

Replicate variability and recovery for direct lysis compared with column-based extraction.

Sample	Titer (RNA extraction) (vg/ml)	Titer (Lysis kit) (vg/ml)	% CV	Average titer (for all replicates) (vg/ml)	% CV (for all replicates)	% recovery against RNA extraction
M – Replicate 1	6.97×10^{8}	5.87×10^{8}	7.76	6.06×10^{8}	5.8	87
M – Replicate 2		6.46×10^{8}	6.40			
M – Replicate 3		5.84×10^{8}	3.07			
N – Replicate 1	9.09×10^{7}	1.44×10^{8}	16.50	1.38×10^{8}	6.3	152
N – Replicate 2		1.42×10^{8}	17.95			
N – Replicate 3		1.28×10^{8}	13.66			
O – Replicate 1	5.58×10^{8}	6.82×10^{8}	8.53	6.36×10^{8}	6.2	114
O – Replicate 2		6.15×10^{8}	6.16			
O – Replicate 3		6.12×10^{8}	5.26			
P – Replicate 1	5.02×10^{7}	8.67×10^{7}	19.32	9.67×10^{7}	8.9	193
P – Replicate 2		1.01×10^{8}	24.43			
P – Replicate 3		1.02×10^{8}	20.02			
Q – Replicate 1	1.64×10^{7}	5.22×10^{7}	3.28	5.15×10^{7}	4.5	313
Q – Replicate 2		5.34×10^{7}	5.95			
Q – Replicate 3		4.90×10^{7}	3.47			

LV: lentiviral.

process, as seen in Table 1. Recovery values ranged from 90–171% relative to the two-step workflow, indicating that direct lysis combined with one-step RT-dPCR consistently achieved genome recovery equal to or greater than that of the two-step workflow.

A second comparison evaluated the direct lysis workflow against silica membrane-based RNA extraction using an identical one-step RT-dPCR readout across another six samples, G–L. Across the panel of test samples, direct lysis produced higher titers in most cases, although

individual sample-dependent differences were observed, as shown in Table 2. Recovery values ranged from 59–349% relative to extraction, indicating that the two methods can differ considerably in yield depending on sample composition and matrix effects. These results highlight the importance of understanding how upstream sample characteristics influence RNA recovery and quantification.

An additional dataset assessed variability within each workflow by measuring replicate titers across five sample types (M–Q), each with three replicates, shown in Table 3. For direct lysis, replicate measurements produced low coefficients of variation across a range of viral titers and matrices. Average titers calculated across replicates remained consistent, and recovery relative to extraction was stable within each sample set. These data indicate that the direct lysis method supports reproducible quantification with minimal variability across technical replicates.

Together, these results demonstrate that the CGT Lentivirus Lysis Kit combined with one-step RT-dPCR provides a reproducible and efficient alternative to silica membrane-based extraction workflows while maintaining or increasing measured viral genome titers in most tested samples, with some sample-dependent differences in recovery.

SUMMARY

dPCR provides a consistent framework for measuring key attributes of LVVs used in *ex vivo* engineered cell therapy workflows. The integrated workflows described combine assays targeting lentiviral regulatory and packaging elements, genomic reference loci, and sequences indicative of replication competence, supporting the absolute quantification of vector copy number, genome-containing particles, and replication-competent species.

A direct-lysis approach for lentiviral RNA preparation reduces handling requirements relative to extraction-based methods while maintaining or improving measured genome titers across concentrated material and process supernatants. Validation datasets demonstrate broad assay linearity, accuracy across simulated VCN levels, stable quantification at high background DNA concentrations, and low variability across replicates.

Independent evaluation at Niba Labs confirmed that one-step RT-dPCR can provide higher viral genome recovery than two-step workflows, and that direct lysis frequently yields higher titer than column-based RNA extraction. Together, these findings support the use of a streamlined dPCR workflow for lentiviral vector characterization and quality control activities in cell and gene therapy development.

Q&A


David Dobnik (left), **Miroslav Vranes** (right)

Q Which assay was used for lentivirus quantification?

DD Lentiviral quantification was performed using an in-house GFP-based assay. A side-by-side comparison with QIAGEN's assay demonstrated

equivalent performance. Due to familiarity with the in-house method and its use in previous experiments, it was selected for ongoing quantification.

Q What is the recommended approach for RNA extraction when performing lentiviral quantification?

DD Due to its simplicity, the lysis kit is generally recommended. However, if highly purified RNA is required for downstream applications beyond dPCR, a conventional RNA purification workflow may be more suitable.

Q Has the QIAcuity RCL quantification kit been compared with cell culture–based methods for RCL testing?

MV Comparative studies with cell culture–based assays or other RCL detection methods have not yet been conducted, however, they are being planned in collaboration with external partners.

Q Why are lentiviral titers higher in direct lysis samples compared with those obtained using sample preparation workflows?

MV Higher titers in direct lysis samples likely reflect incomplete recovery during conventional sample preparation. When sample preparation workflows do not recover all viral material, direct lysis may yield higher RNA quantities and correspondingly higher titers.

Q Why do titers differ across the three lentiviral-specific targets?

MV Differences among targets are believed to arise from secondary structural features of the viral RNA genome. These structural differences lead to consistently lower quantification for LTR, higher values for Psi, and the highest values for RRE. This pattern is observed across both direct lysis and conventional sample preparation.

Q If the QIAamp RNA Viral Mini Kit yields lower titers than direct lysis, should only the lysis kit be used for lentiviral samples? Can the direct lysis kit be applied to other enveloped RNA viruses? When is sample preparation preferred over lysis?

MV The direct lysis kit has not yet been evaluated with other RNA viruses, so broader applicability has not been confirmed. Both the lysis approach

and conventional sample preparation are compatible with lentiviral quantification. In general, conventional sample preparation yields slightly lower RNA amounts. In scenarios involving highly inhibitory samples, although such samples have not yet been observed, sample preparation may offer an advantage. For rapid, straightforward workflows, direct lysis is a suitable option and provides flexibility for DNase treatment or dilution steps at multiple points in the process.

Q Why do thresholding levels differ between samples, and what criteria define appropriate thresholding?

MV Thresholding is automatically performed by the software, which typically eliminates the need for manual adjustment. The automated thresholding criteria are designed to ensure consistent and appropriate application across samples.

Q The lysis kit handbook states that long-term storage of lysates is not recommended. Is information available on degradation rates at –80 °C, and can storage stability be improved?

MV Studies on long-term lysate stability are ongoing, and the handbook will be updated once sufficient data are available. Current tests indicate that lysates stored within the PCR setup, such as in pre-plates or nanoplates, remain stable for at least overnight storage at room temperature. Data for long-term storage are not yet available, but experiments are underway to assess stability and potential mitigation approaches.

AFFILIATIONS

Ruth Klaver PhD, Senior Global Product Manager, QIAGEN, Hilden, Nordrhein-Westfalen, Germany

Miroslav Vranes PhD, Associate Director of R&D and dPCR Applications Development, QIAGEN, Hilden, North Rhine-Westphalia, Germany

David Dobnik PhD, Co-founder and Chief Scientific Officer, Niba Labs, Ljubljana, Slovenia

AUTHORSHIP & CONFLICT OF INTEREST

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Sample to Insight

Advancing cell dissociation in bioprocessing: a novel, high purity trypsin-like enzyme for GMP workflows

Tjebbe Van Der Meer, Head of Product Management, c-LEcta GmbH (Operator of the Kerry Biotechnology Centre and part of the Kerry Group)

Cell & Gene Therapy Insights 2026; 12(1), 13 · DOI: 10.18609/cgti.2026.003 ·

Reliable cell dissociation is essential for biopharma manufacturing, especially under GMP conditions. This poster introduces a novel GMP-grade recombinant enzyme of fungal origin, designed to enable gentle and consistent cell dissociation across a wide range of applications.

INTRODUCTION

Efficient and reliable cell dissociation is critical for bioprocessing workflows including viral vector and vaccine manufacturing, stem cell processing, and cell banking. Rising regulatory expectations and the need for animal origin-free reagents have driven the development of recombinant trypsin-like enzymes of fungal origin. These enzymes offer gentler dissociation compared to porcine trypsin, preserving cell viability and phenotype in sensitive systems such as induced pluripotent stem cells (iPSCs) and production cell lines.

ENZYME PERFORMANCE COMPARISON

To assess enzyme performance, CellTrypase was evaluated across a range of standard and advanced cell types, using the current industry standard as a control. Enzyme purity was assessed by high-performance liquid chromatography (HPLC), with overlapping peaks demonstrating similarity between the two enzymes **(Figure 1)**. However, the industry standard exhibited a broader peak base with several smaller peaks, indicating the presence of impurities. To ensure consistent performance, each CellTrypase batch will be released only when a purity of ≥95% is achieved, as this high purity aids specific activity and consistent performance in sensitive culture workflows.

To assess the suitability of CellTrypase for routine cell passaging in biopharmaceutical manufacturing, four commonly applied adherent cell lines were tested in-house. No significant performance differences were observed between CellTrypase and the industry standard across CHO-K1, HEK 293, MDCK, and Vero cells **(Table 1)**. These results support the use of the enzyme as a direct replacement for conventional trypsin solutions.

DISSOCIATION OF 2D COLONIES OF HUMAN iPSCS

To evaluate applicability of CellTrypase in advanced and sensitive cell systems, its performance was compared with the industry standard in human iPSCs cultured as 2D colonies **(Figure 2A)**. In these cultures, CellTrypase enabled faster cell detachment than the industry standard without compromising cell integrity (5 versus 7 minutes). Viability remained high and comparable between both enzymes **(Figure 2B)**.

Preservation of pluripotency is a critical requirement in stem cell workflows. Flow cytometric analysis confirmed that pluripotency marker expression following enzyme dissociation exceeded the predefined acceptance threshold of 75% across the marker panel **(Figure 2C)**. These data demonstrate that the enzyme supports gentle dissociation without altering cell phenotype.

SUMMARY

CellTrypase is a new trypsin-like enzyme of fungal origin for the dissociation of cells in biopharmaceutical workflows. In this study, CellTrypase delivered performance equal to or better than the current industry standard across key production cell lines and iPSCs. Furthermore, HPLC analysis confirmed a significantly higher purity profile for GMP-grade CellTrypase compared to the GMP-grade industry standard. With a defined purity specification of ≥95% CellTrypase sets a new standard for consistent dissociation of sensitive cell systems.

Figure 1. Purity of 10× concentrated stock solutions of GMP-grade CellTrypase (red) and the current industry standard trypsin-like enzyme (grey) as measured by HPLC.

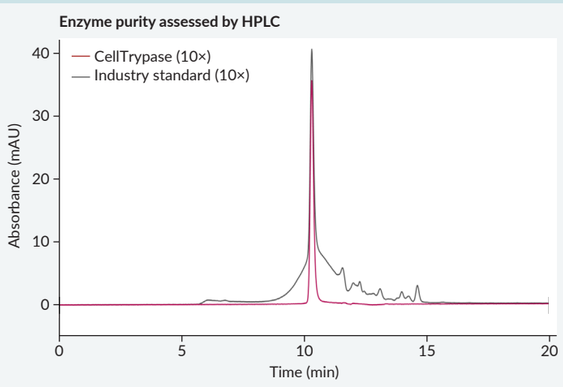
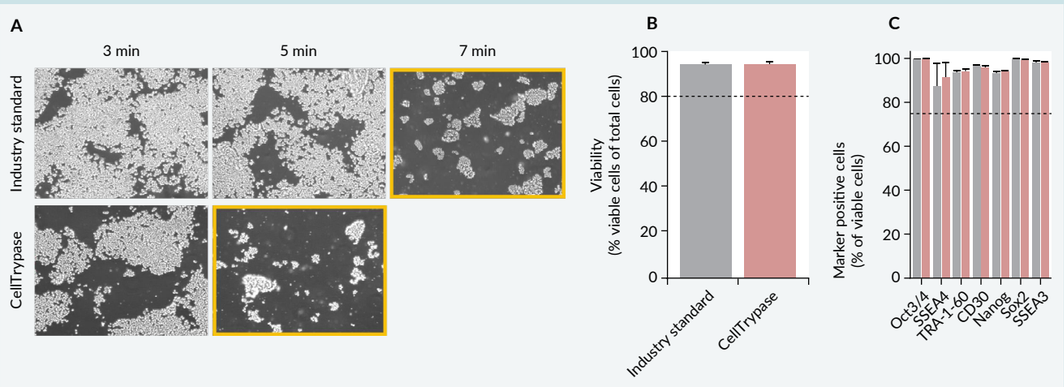


Table 1. Summary of dissociation performance of key biopharma cell lines using 1× CellTrypase. Blue tick means CellTrypase matches or exceeds the performance of the current industry standard enzyme.

	CHO-K1		HEK 293		MDCK		Vero	
	Industry standard	Cell Trypase	Industry standard	Cell Trypase	Industry standard	Cell Trypase	Industry standard	Cell Trypase
Dissociation time (mm:ss)	02:30	02:25 ✓	02:46	02:27 ✓	25:23	23:32 ✓	03:59	04:07 ✓
Viability (% of total)	97%	98% ✓	95%	95% ✓	99%	99% ✓	99%	99% ✓
Yield (% of industry standard)	100%	106% ✓	100%	100% ✓	100%	102% ✓	100%	103% ✓
Doubling time (hh:mm)	15:17	14:47 ✓	27:10	26:55 ✓	19:09	18:52 ✓	19:45	19:16 ✓

Figure 2. (A) Dissociated, 2D-cultered iPSCs using either a 1× ready-to-use solution of CellTrypase or the industry standard enzyme; yellow boxes indicate dissociation times. (B) Viability post-dissociation using the industry standard versus CellTrypase. (C) Pluripotency marker protein expression post-dissociation measured by flow cytometry.



Tjebbe van der Meer is a commercial specialist with more than 20 years of experience in biomanufacturing innovation. His background includes process modelling, monoclonal antibodies and viral vector production processes. In his current role he leads the biopharma enzyme products of c-LEcta, which target the vaccine and cell and gene therapy applications. Prior to joining c-LEcta, Tjebbe held several product management and sales positions at Sartorius in Göttingen, Germany.

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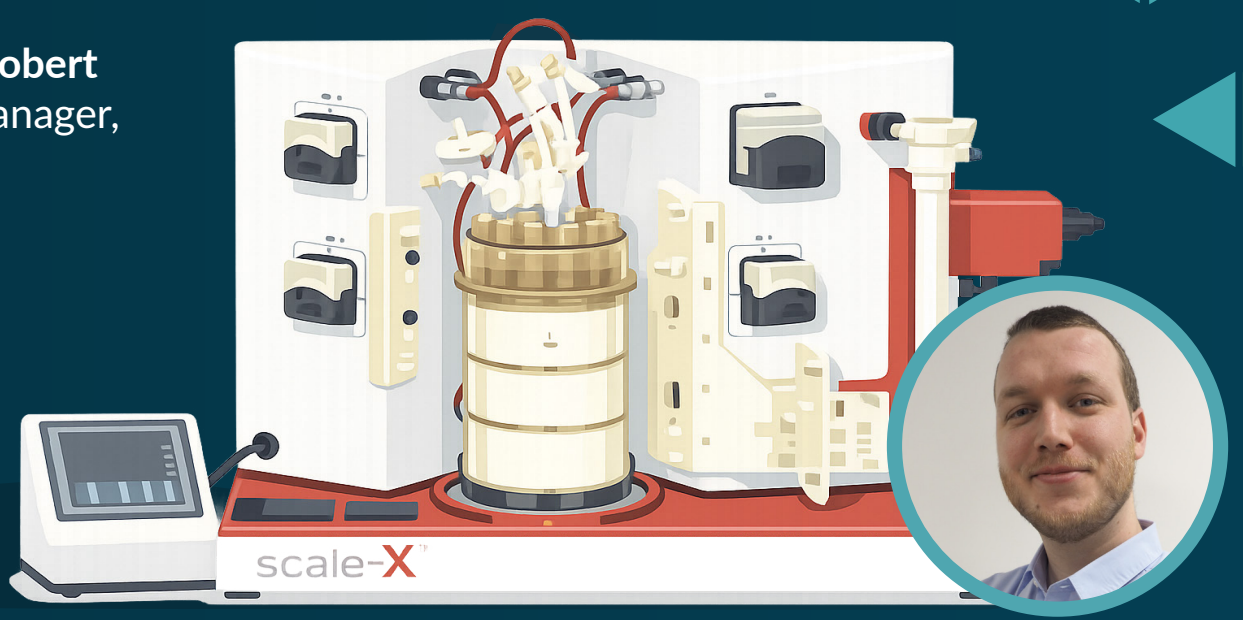

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