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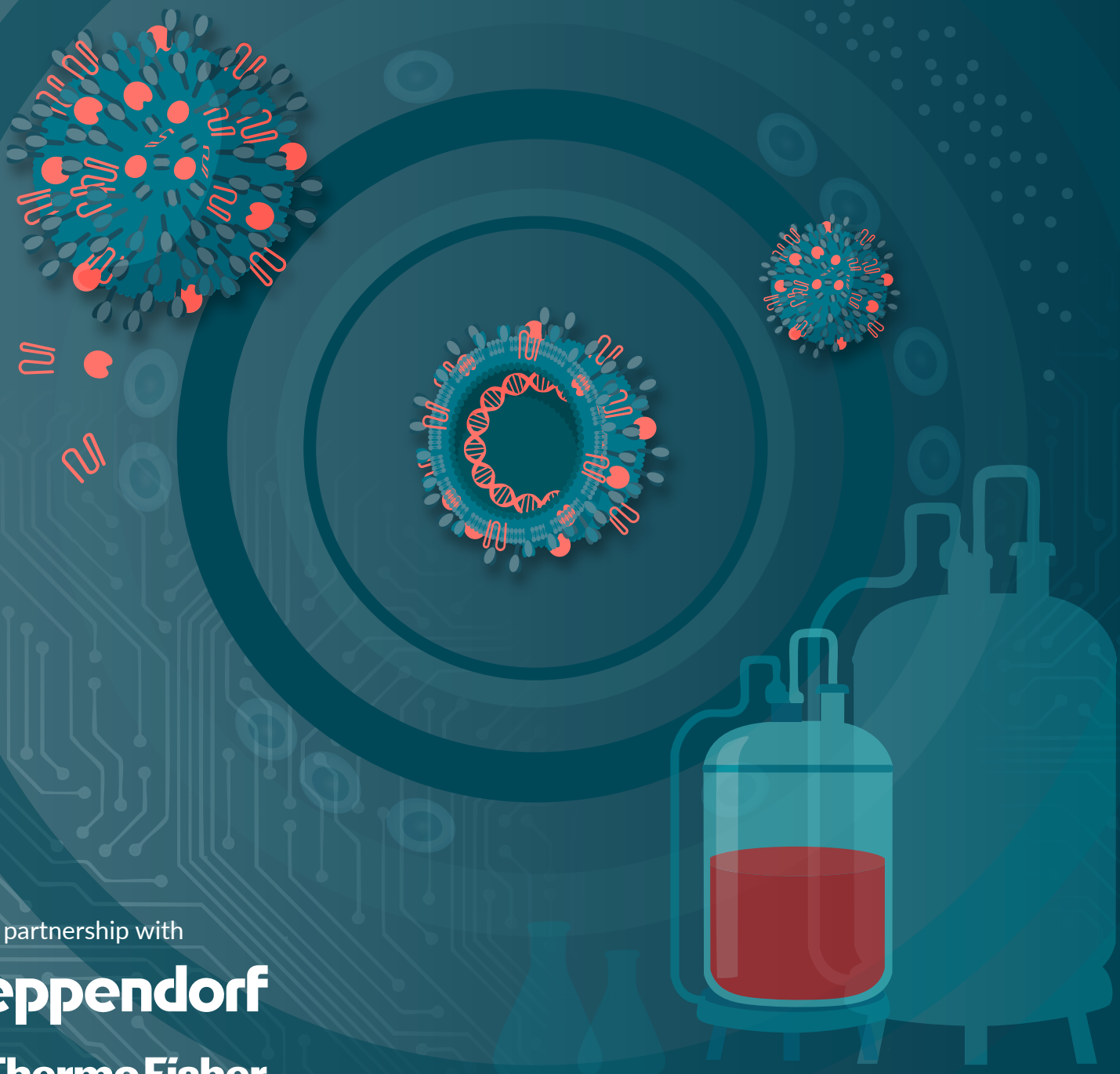
CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

Scale-up/-out of cell and gene therapy manufacturing



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EXPERT INSIGHT

Tumor-infiltrating lymphocyte therapies: personal, powerful, with possibilities for improvement

Sérgio T Ribeiro and Therese Choquette

Tumor-infiltrating lymphocyte (TIL) therapy is one of the most promising adoptive cell therapies (ACT) for solid tumors, with a personalized and multi-antigen recognition of tumor cells without requiring genetic modification. Despite clinical success, particularly in melanoma, the field of TIL therapy faces adoption challenges related to manufacturing complexity, high costs, and a limited understanding of the requirements to overcome the hostile tumor microenvironment (TME) that impairs TIL efficacy. This article presents a perspective on TIL manufacturing, addressing aspects such as tissue quality, logistics, labor-intensive and manual workflows, and analytical testing, while exploring how emerging technologies could provide new opportunities for improvements. Through the implementation of intelligent, automated bioreactors with process analytical technologies (PATs), combined with advanced tumor and TIL characterization tools and a better understanding of TIL critical quality attributes (CQAs), the process consistency, potency assessment, and scalability can be significantly improved. The integration of multi-omics data, real-time process monitoring, and deep product characterization has the potential to make TIL therapies more robust, accessible, and effective across diverse tumor types.

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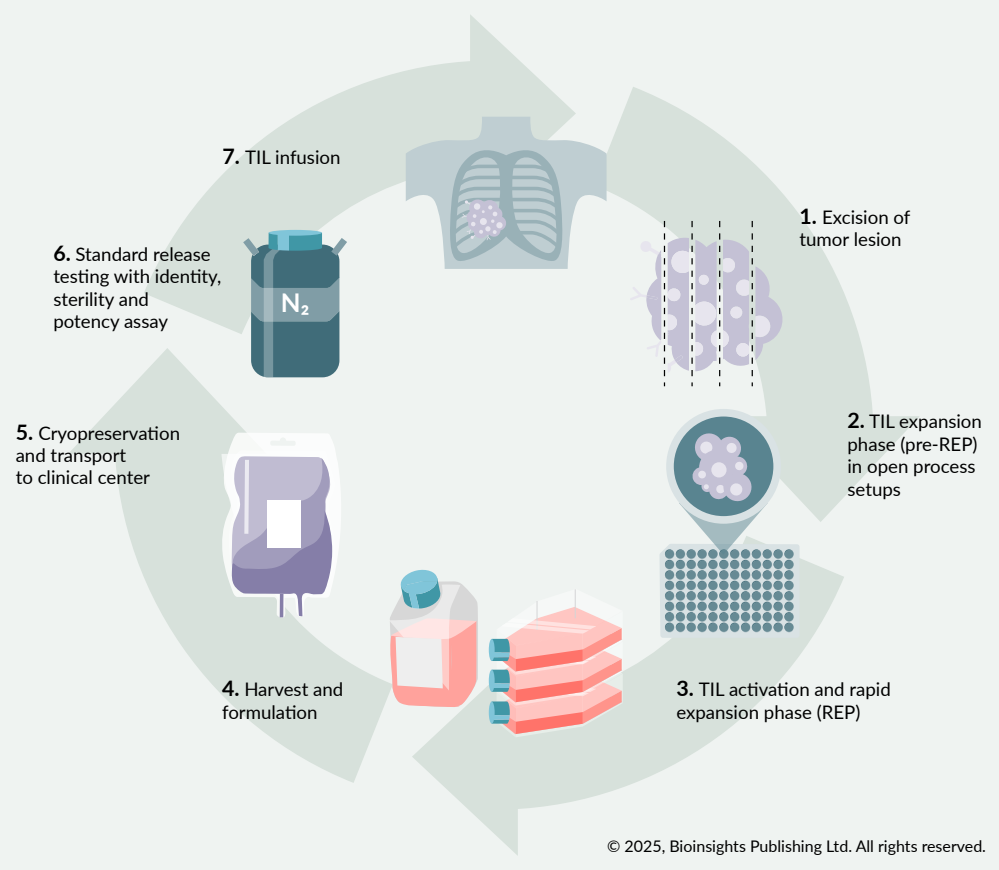
WHAT MAKES TIL THERAPY SO SPECIAL?

As of today, tumor-infiltrating lymphocyte (TIL) therapy is the most successful adoptive cell therapy (ACT) for solid tumors [1]. TIL therapy is manufactured using the patient's tumor, which contains TILs. These cells are expanded *ex vivo* and re-infused as a personalized autologous TIL therapy for solid tumors (Figure 1). TILs comprise a polyclonal T cell repertoire that recognizes both shared tumor antigens and neoantigens, reducing the need for genetic modifications. The polyclonal, multi-antigen reactivity of TILs is a major advantage compared with single-target strategies such as CAR-T cells or monoclonal antibodies, as it reduces the

FIGURE 1

Overview of the standard manufacturing workflow for tumor-infiltrating lymphocytes.



risk of tumor escape through antigen loss. To further increase efficacy, ongoing studies are investigating genetically modified TIL products to improve their survival and function within the hostile tumor microenvironment [2].

TIL therapy represents a very promising advancement in cancer treatment, particularly for patients for whom immunotherapies such as anti-PD-1, anti-PD-L1, and anti-CTLA4 have demonstrated limited efficacy [3]. Initially tested in clinical trials at NIH by Steve Rosenberg and his team in the late 1980s [4], TIL therapy has since shown clinical promise, most notably in advanced melanoma, and the regulatory approval of Amtagvi™ from Iovance Biotherapeutics highlights its therapeutic and commercial potential. Recent studies further support TIL's therapeutic efficacy against other malignancies, including non-small cell lung cancer, cervical cancer, and head and neck squamous cell carcinoma [5].

Despite encouraging outcomes in solid tumors, the broad adoption of TIL therapies remains constrained. Like other autologous treatments, personalized TIL therapy, one batch—one patient, comprises logistical and operational limitations. This contrasts with other immunotherapies, such as monoclonal antibodies, where one batch can treat multiple patients. In addition, some of the requirements are unique to TIL manufacturing, including the need for sufficient viable tumor tissue for manufacturing, a semi-manual and prolonged production process (compared to CAR-T therapies), and the requirement of

high cell numbers for the final product [1]. Importantly, developing a potency test to evaluate TIL functionality is challenging due to the absence of a single target antigen (as for CAR-T cells) and the diversity of patient-specific neoantigens. All together, these complexities contribute to the high cost for TIL therapies and the need for specialized infrastructure on the clinical side to support tumor tissue collection.

However, those working in the field and committed to improving the future of TIL therapies are fully focused on finding solutions. Here, we share our perspective and thoughts on what can be done in manufacturing to make TIL therapies more attractive to develop and more accessible to patients.

THE QUALITY OF THE TUMOR TISSUE DICTATES THE QUALITY OF THE PRODUCT

Unlike other T cell-based therapies, such as CAR-T, which begin with peripheral blood, TIL therapies rely on solid tumor tissue obtained from the patient to manufacture the final product [1]. Most often, there is no easy access to harvest the tumor, a simpler biopsy might be impossible, and surgery may be the only viable option for getting the tumor. Once the tumor is collected by the surgeon, normal tissue is trimmed off the tumor, and a small tumor piece is sent to the pathologist for standard analysis. The harvested tumor is transported in cold temperature to the manufacturing site for TIL manufacturing. The time spent collecting, processing, and transporting the tumor to the manufacturing site, the transport media and temperature variations impact the condition and quality of the T cells in the tumor sample [6]. Altogether, patient-specific and logistical variables are key external factors that introduce heterogeneity in the starting material and impact the composition and quality of the final TIL product.

In addition to these external parameters, intrinsic biological and tumor-associated factors also impact TIL manufacturing outcomes. Similar to the blood-derived starting material in CAR-T and TCR-based therapies, the quality of TILs is affected by the patient's overall condition, prior treatments, and disease status. Tumors vary widely, not only by tissue of origin and between patients with the same tumor type, but also within the same or different lesions in the same patient. For example, variations may include tumor size, degree of necrosis, volume of viable tissue, content of adipose tissue, and composition of the TME, including TIL density. Importantly, prior treatments such as radiation can reduce TIL viability and functionality, making irradiated or heavily pretreated tumors less suitable starting material [7,8]. These findings (reviewed elsewhere [7]) highlight that tumor tissue collected for manufacturing is not homogenous, which creates challenges for predicting manufacturability, comparability studies, as split material may contain different cell populations and therefore generate products with slightly different quality attributes [7,9,10]. It also makes it difficult to standardize the tumor collection procedure and to provide a starting material with defined attributes for the manufacturing process.

Given the importance of starting material quality, the use of fresh tumor tissue is generally preferred as it better preserves cell viability and recovery, supporting robust *ex vivo* expansion and functional TIL products. In contrast, cryopreservation of the tumor, while operationally convenient, has been shown to be difficult to optimize [11]. It reduces cell viability and recovery, thereby affecting TIL expansion and potentially increasing the risk for manufacturing failure [12]. However, the use of fresh tumor tissue introduces logistic complexity, as the time between tissue collection and the start of manufacturing is limited due to its impact on cell quality and viability. Fresh

tumor tissue requires fast analysis by the pathology department, efficient coordination with surgical schedules, and immediate transport to the manufacturing site for processing.

Decentralized manufacturing of TIL therapy facilitates the use of fresh starting material by being located close to hospitals and patients [13,14]. An additional way to ensure high-quality samples is to reduce tumor assessment time by equipping surgeons and pathologists with first-in-class tools for real-time assessment during tumor collection. Where surgeons can be guided with information about the presence of TILs, fat content, necrosis, and other factors, to facilitate the collection of high-quality tumor tissue for manufacturing. This allows the surgeon to adjust and collect tissue from different tumor areas as needed. Since time is critical for best-in-class TIL therapy, combining real-time assessment with transport to the nearby decentralized manufacturing site offers the shortest interval, helping to preserve optimal starting material.

A COMPLEX MANUFACTURING PROCESS WITH POSSIBILITIES FOR IMPROVEMENTS

The manufacturing process of TIL therapies is complex and challenging [15], involving fragmented process steps, with transfers of cells between vessels, particularly during early stages of product manufacturing [5,16]. This can result in production failures, contaminations, long manufacturing turnaround times, and difficulties with product transportation. Despite these hurdles, momentum is building, and the TIL manufacturing success rate is typically above 90%, and next-generation strategies could further reduce failures and costs. For genetically engineered TIL products, efficient transduction using retroviral vectors has proven difficult, however, efficient genetic editing approaches such as CRISPR and TALENs are being tested to improve persistence and reduce exhaustion of infused TILs [5].

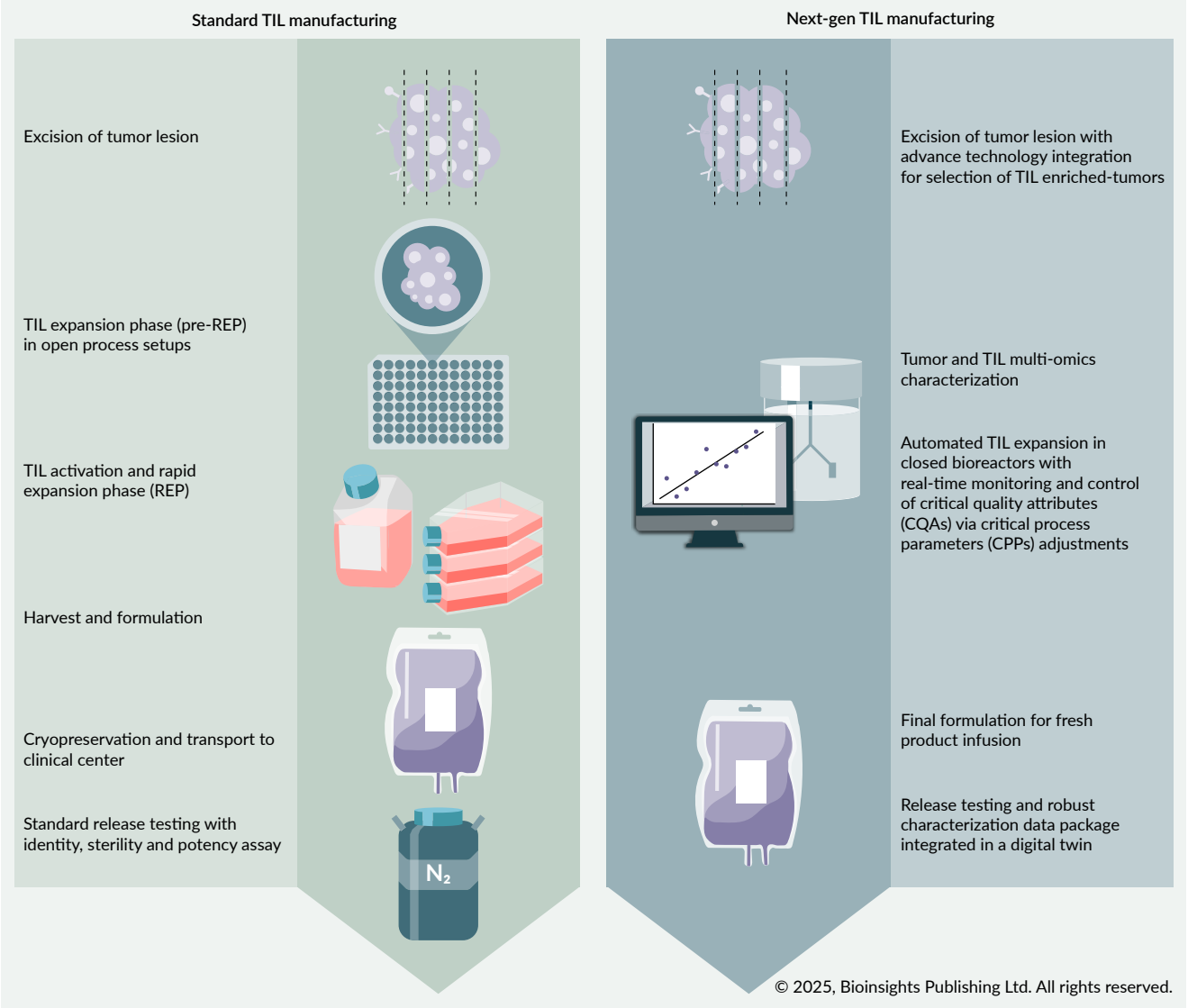
The manufacturing process for TIL therapy currently relies on feeder cells, primarily PBMCs from healthy donors, to support TIL expansion. While feeder cells work effectively, the donor variability leads to non-standardized process conditions between batches. In addition, the PBMC feeders contribute to the increased cost of goods, as they must be GMP-compliant and qualified for release. Moreover, the exact mechanism by which feeder cells support TIL expansion is not fully understood, highlighting the need for further research studies to develop robust alternatives that can be implemented in the next-generation manufacturing process (Figure 2).

Manual handling during manufacturing and the use of semi-closed systems introduces risks of contamination and may impact product characteristics, including T cell phenotypes, cellular functions, and overall potency. The implementation of automated bioreactors and closed systems able to handle tumor fragments as starting material can minimize process-related variability, improve product attributes, and accelerate TIL manufacturing [13,14,17,18]. Examples of closed and automated platforms already available or under development for TIL manufacturing include the X3® (ADVA), IRO® (Oribiotech), Sefia™ (Cytiva), CliniMACS Prodigy® (Miltenyi Biotec), Cell Shuttle™ (Cellaris), and other platforms based on G-Rex® (Wilsonwolf) systems.

Scalable and reproducible production, tailored to the heterogeneous starting material, is facilitated by integrated real-time monitoring with advanced process and analytical technologies (PAT), such as temperature, pH, dissolved oxygen (DO), cell metabolites, and media nutrient levels. These tools not only enable intervention and control of batch runs, when necessary

FIGURE 2

Side-by-side comparison of standard and conceptual next-generation TIL manufacturing processes with suggested improvements and integrated analytical strategies.



but can also optimize the cell expansion from heterogeneous starting material, such as tumor fragments. Sensor-driven manufacturing platforms and microfluidic technologies hold promise for providing deep process insights, reducing costs, and potentially improving quality attributes and consistency of the product.

Altogether, significant efforts and investments are being directed toward the development of closed, automated, and sensor-integrated platforms tailored to the manufacturing of ACTs [11–14,17–20]. These innovations promise to significantly improve the manufacturing process for TILs in multiple ways. Not only will they decrease risks by minimizing the need for manual sampling during the process and enhance overall product quality, but they will also facilitate training, technology transfers, and comparability studies across manufacturing sites, and ultimately reduce cost barriers for broader implementation of TIL therapies.

ANALYTICS, WE ONLY KNOW WHAT WE MEASURE

As with any ACT, release testing of the final product is the last step before quality review, approval, and release for transport to the patient. Two of the most debated tests in this space are the required sterility and potency testing. Currently, there is no approved rapid sterility test for final product release that delivers results within 1 day. The compendial (pharmacopeia) methods take up to 14 days to generate the result. While some approved non-compendial rapid sterility tests are available, they still require 5–7 days for completion. In practice, autologous cell therapies can be released prior to final sterility results using interim strategies such as Gram stain testing, with conditional release under a defined clinical risk-management plan [21], which increases costs and resource requirements. Nevertheless, there remains a critical need for robust and validated rapid sterility technologies that can deliver results instantly. Adoption of such methods would substantially reduce dependence on interim release strategies, shorten timelines, mitigate risk, lower costs [22], and potentially enable infusion of fresh products and facilitate the administration of life-saving treatments with limited shelf life.

Potency testing is also a major focus of debate, frequently discussed in scientific conferences, publications, and in regulatory panels due to the complexity of this analytical requirement for ACT product release [23,24]. It is especially challenging for TIL therapies due to the vast repertoire of antigens recognition, which are specific for unique neoantigens and tumor-specific antigens. Therefore, no cell lines are currently available that express the full range of relevant antigens and are specific for TILs. While tumor cells could, in principle, be extracted from the collected tumor tissue and used as target cells, this approach would be limited by low reproducibility, highly resource-intensive and logistically complex, making it unlikely to be used in a method suitable for QC, GMP, or validation purposes. So, what can be used as potency assay for a TIL product? There is no definitive answer; each manufacturer must consider the mechanism of action (MoA) and the critical quality attributes (CQA) to develop the most appropriate potency assay for their product. The review by Betof Warner et al. suggests a potency matrix approach to provide a comprehensive picture of both potency and identity [15]. This matrix may incorporate multiple functional attributes, such as quantitation of IFNg secretion in response to antibody-coated beads or co-culture with a target cell line, combined with the identification of specific T cell phenotypes.

The release testing ensures that the product meets the required attributes for identity, potency, and safety. However, to fully capture and understand the complexity of the living product and its therapeutic potential, additional characterization methods are needed. The characterization testing is performed in non-GMP conditions using analytical assays that are scientifically sound and fit-for-purpose. This characterization must begin during product development and as early as possible, at the stage when a comprehensive understanding of the product is essential [24].

With the aim of achieving a highly efficacious therapeutic product, it is essential to extensively characterize not only the final product, but also the starting material, the tumor tissue, from which the final product is directly derived. It is crucial to collect enough tumor material while also capturing the heterogeneity within the sample. The manufacturing processes using tumor fragments lack information about the number of TILs at the start of the process, whereas processes that start from digested tumor tissue can quantify the starting TIL population. There are pros and cons to both alternatives, which need

to be evaluated and defined during process development. The assessment performed by the pathologist during tumor tissue collection is crucial, and the information collected can integrate as an important part of the characterization package. Therefore, close collaboration between the clinical, translational, and analytical departments is essential to ensure that all information and insights from the pathology assessment are fully captured and incorporated into the product's characterization.

Data collected during the manufacturing process (in-process testing) is just as important as testing other samples, such as tumor tissue or final product. This information is essential for identifying and establishing critical process parameters (CPP) [12–14,17,19]. One bottleneck in TIL manufacturing is the low number of cells and their sensitivity to disturbance, making cell sampling and testing difficult. Using bioreactors equipped with PATs from the very first step of the TIL process can generate critical information, potentially eliminating the need for early in-process sampling. This improves manufacturing control and reduces associated risks. Data gathered from in-line and on-line bioreactor monitoring is essential for effective process control.

New technologies using multimodal and smart automation, integrated sensors during cell expansion, and scalable -omics workflows are powerful tools for characterization of both TIL products and tumor tissue. Transcriptomics, proteomics, metabolic profiling, and an array of different functional assays, combined with clinical and patient-specific data, may offer the insights needed to understand what makes a TIL product efficacious across different indications [7,9,10]. Given the high heterogeneity of tumor tissue and TILs, artificial intelligence (AI), machine learning (ML), and the use of digital twins can help identify key patterns across large datasets. As shown in recent studies applying AI and ML to TIL phenotyping and functional assessments [9,10,13,14], these approaches can complement biological assays by revealing key patterns across large datasets that are not easily detected through conventional analysis.

A STEP CLOSER TO EXPANSION OF TIL THERAPIES FOR MORE PATIENTS

TIL therapy has shown encouraging results in the treatment of solid tumors, but key aspects remain to be understood about TIL products, including which quality attributes of the starting material and final product are critical for manufacturing success, optimal function, and persistence in patients. The translational analysis of manufacturing and characterization data, when integrated with clinical datasets, will support the identification of a specific combination of product and patient attributes associated with optimal clinical efficacy and safety.

We believe that a decentralized manufacturing model close to patients is well-suited for TIL therapies, as it reduces the turnaround time of the manufactured product and the cost of goods. It also eliminates the need for cryopreservation, which negatively impacts the robustness and function of the cells in the starting material and ultimately the final product [19]. With the use of fresh (non-cryopreserved) cells, this model helps preserve cellular function and improve the quality of the TIL product.

The selection and handling of tumor tissue are critical for achieving a high-quality final product. Real-time pre-surgical and intraoperative analyses, combining immune profiling, TIL density, and tissue quality, contribute to an improved manufacturing process and higher-quality TIL products [25]. This requires advanced analytical technologies and close coordination between surgeons and pathologists, who play a critical role in selecting viable, appropriate, and sufficient tissue samples.

Automated and closed manufacturing systems equipped with non-disruptive in-line and on-line PATs enable real-time monitoring of cells in the bioreactor and reduce manual sampling, the risk of contamination, and processing time. In addition, they provide increased control of the process as well as more detailed information about the status of cells during the process. Training operators on these automated systems is more straightforward and facilitates tech transfers and comparability studies.

Also, in the QC lab, fully automated instruments for analytical tests and subsequent data analysis can save resources and reduce costs. These instruments are often cartridge-based and utilize microfluidics, enabling tests to be performed with a low number of cells and with minimal manipulation. This approach reduces the risk for inaccurate results caused by sample handling, such as washing, labeling, and centrifugation. In addition, automation improves overall consistency and reliability, helping to avoid delays in product release caused by investigations and re-tests. The simplified workflows also save time and resources by facilitating analysts training, methods transfer, and comparability studies.

TIL therapy presents a promising treatment option for solid tumors, offering a personalized, multi-antigenic approach without the need for genetic engineering. However, the broad clinical adoption of TIL therapies remains limited, despite favorable clinical outcomes and a high manufacturing success rate (>90%). These challenges are likely due to the complex collection and analysis of the starting material, as well as the inherent complexity of the manufacturing process. Overcoming these barriers through rapid and optimized analytics, streamlined manufacturing, and advanced automation will be paramount for broadening clinical adoption and maximizing therapeutic benefit for cancer patients.

TRANSLATION INSIGHT

Advancing TIL therapy to widespread clinical application requires a focused effort on understanding how to manufacture a robust, highly functional product capable of overcoming the hostile tumor microenvironment. Translational analyses that integrate patient clinical data with the manufacturing data can reveal critical attributes of both the patient and products that underpin optimal clinical efficacy. Integrating tumor profiling, real-time sensor-based process control, and close coordination between clinical, analytical, and manufacturing teams is essential to ensure consistent product quality, enable scalable production, and expand access to TIL therapies for patients with solid tumors.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

COMMENTARY

People, not platforms: the real limit to cell and gene therapy scale-up in Europe

Anji Miller, Eleuterio Lombardo, Janine Kirby, Rosie Lindup, Nicola Ambler, Rebecca Ludwig, and David Morrow

In the race to develop cell and gene therapies (CGT), the prevailing narrative has focused on the promise of cutting-edge science, robust clinical pipelines, and the emerging potential of digital biomanufacturing. But behind the glossy headlines and billion-euro investments lies a less glamorous, more human truth: no matter how brilliant the science or how sophisticated the platforms, innovation will falter without a skilled and sustainable workforce. This is not an abstract concern. Stories continue to circulate about promising well-funded biotech that faced failure not due to a science but a people problem. In simple terms, there were not enough qualified personnel to scale up their process and manage their development. This scenario is playing out across the CGT ecosystem, not only in Europe but globally. As countries invest heavily in biotechnological infrastructure and translational research, a critical bottleneck is emerging not in ideas or capital, but in human capacity. Despite an array of training initiatives, demand for skilled professionals continues to outpace supply. If left unresolved, this imbalance could be the single greatest threat to CGT scalability over the next decade. Furthermore, training initiatives alone will not address the skills gap in this growing CGT ecosystem. Finding the right ways and incentives to attract and retain this newly skilled workforce within this space must also be addressed.

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UNMASKING THE GAPS: WHERE WE ARE FALLING SHORT?

The workforce crisis in CGT development and delivery is multifaceted. Some of the most urgent shortfalls are technical. According to a 2023 Nordic industry survey, 74% of CGT employers report deficits in GMP manufacturing and compliance skills including aseptic technique, cleanroom behavior, and bioreactor operation echoing similar challenges as mentioned above [1,2].

In the USA, the largest gaps lie in QA/QC and regulatory CMC documentation

skills are essential for trial progression and product release [3].

Even more daunting is the trajectory of demand. The UK headcount in the CGT sector doubled from 3,033 in 2019 to 6,232 in 2023 and is projected to exceed 10,000 by 2028 [4]. Nordic projections foresee a 33% workforce shortfall by 2035 under moderate growth scenarios [2] and that is before we consider the digital layer. Digital transformation is reshaping advanced therapy medicinal product (ATMP) development, requiring entirely new competencies. The UK's Cell and Gene Therapy Catapult recently identified over 130 digital knowledge-skills-behaviors (KSBs) needed to support automation by 2028, from Manufacturing Execution System (MES) and Laboratory Information Management System (LIMS) integration to real-time analytics and AI-based process control [5]. Yet fewer than 15% of biotech leaders feel ready to lead digitalized teams [6]. Meanwhile, demand for bioinformaticians and multi-omics experts is expected to grow by 184% by 2026 [7].

Just as critical and arguably more overlooked are the transferable skills required in this space. CGTs operate at the intersection of science, regulation, commercialization, and care. Yet most training models remain siloed, leaving professionals unprepared for interdisciplinary collaboration, technology development, innovation management or leadership. While 70% of life science firms recognize a leadership gap, only 11% feel adequately prepared [8].

THE FIELD IS GROWING FASTER THAN OUR INSTITUTIONS

These challenges are compounded by systemic gaps in training and educational infrastructure. The UK is widely recognized as a global leader in advanced therapy translational research, especially in the development and clinical translation of cell and gene therapies. CGT-related research is accelerating, leading to more preclinical and clinical studies that require trained staff, robust infrastructure and coordinated institutional support. But the workforce pipeline is not keeping pace, especially for mid-career healthcare professionals and scientists looking to upskill.

Pharmacists, for instance, have directly expressed a desire to better understand the science behind these therapies, but currently lack structured opportunities to do so. Many would like to pursue lifelong learning, but there is a glaring scarcity of part-time and online programs that would allow them to learn while continuing to work. Financial constraints and sustainability concerns make some higher education Institutions reluctant to develop such flexible pathways, particularly without external funding or long-term guarantees.

There are precedents however for how this might be addressed. The Genomics England-funded training model significantly advanced upskilling in genetics and genomics across the National Health Service (NHS). Ring-fenced investment enabled the rapid development of accredited taught and online courses [8]. As gene and cell therapies often emerge directly from genomics research, a similar strategy could catalyze the development of a future-ready CGT workforce.

SO, WHAT'S BEING DONE?

There are initiatives making an impact. Over 30 dedicated training programs now support the CGT workforce across the UK and Europe. The Advanced Therapies Apprenticeship Community (ATAC) has enrolled over 300 apprentices [9], and the Advanced Therapies Skills Training Network (ATSTN) has trained nearly 4,000 people in GMP and digital technologies [10].

In the USA, the NIIMBL–WE-BET initiative has scaled ATMP curricula nationally [11]. In 2023, The Alliance for Regenerative Medicine (ARM) recently

published a report providing a landscape overview, gap analysis and recommendations for the workforce needed for sustainable biomanufacturing of CGTs in the USA [12]. In June 2025, the US FDA convened its first Cell & Gene Therapy Roundtable, bringing together regulators, researchers, and patient advocates to address systemic barriers [13].

In the UK, the Innovation Hubs for Gene Therapies (IHfGT) are a UK-wide network of state-of-the-art facilities located in London (King's College London, University College London [UCL]), Bristol (NHS Blood & Transfusion [NHSBT]), and Sheffield (University of Sheffield [UoS]). Funded by LifeArc and the Medical Research Council (MRC), with additional support from the Biotechnology and Biological Sciences Research Council (BBSRC), the hubs are designed to bridge the gap between laboratory research and clinical trials by addressing key challenges in the field, namely, GMP (good manufacturing practice)—grade vector manufacturing, regulatory compliance, and translational expertise. The hubs provide academic researchers with access to resources and support for manufacturing GMP-grade vectors and navigating translational and regulatory pathways by offering capabilities for GMP viral vector manufacturing, translational support, and regulatory advice, thereby addressing a major barrier to academic-led development of gene therapies. This integrated support model is helping to bring innovative treatments closer to patients more efficiently and effectively [14]. A key component of this initiative is the IHfGT Skills and Training Group. This group aims to address the sector's critical skills gap by offering targeted training opportunities, the group is working to build the workforce needed to sustain and grow the gene therapy sector. The group's recent report outlines the unique IHfGT model and presents findings from extensive stakeholder engagement and survey activities. The report highlights current challenges, maps existing capabilities, and identifies priority areas for skills development. It serves as a strategic guide for shaping future training initiatives and ensuring the UK remains at the forefront of gene therapy innovation [2].

The IHfGT Skills and Training Group offers:

- Full-time MScs in Advanced Cell and Gene Therapies (UoS) and Manufacture and Commercialization of Stem Cell and Gene Therapies (UCL) and Applied Transfusion and Transplantation Science (UWE/NHSBT)
- Short courses such as Cell and Gene Therapy Bioprocessing (UCL), Continuous Improvement into Cell and Gene Therapies (NHSBT) and Management of Clinical Services (UWE/NHSBT)
- Online courses like Manufacturing ATMPs (NHSBT) and Gene Therapy: Development of Preclinical and Clinical Studies and Associated Regulatory Processes (UoS)

Another key initiative is the RESILIENCE UK Medicines Manufacturing Skills Centre of Excellence, a partnership between the University of Birmingham, UCL, Heriot-Watt University, Teesside University and Britest Limited. The RESILIENCE Centre supports the UK medicines manufacturing workforce via three workstreams including core materials for training and outreach, cutting-edge digital training, and accelerator programs (for all career entry points). The RESILIENCE Centre has received £4.5 million in funding from the UK government, as part of a broader £1.1 billion 'future tech' skills funding package announced in 2024. So far, the RESILIENCE Centre has trained over 1,200 students and industry professionals,

onboarded almost 200 organizations, and provided over 20,000 hours of cumulative training time. Cell and Gene Therapy Catapult [5] and ATSTN [1] also offer resources, including in-person training, and centralized hubs for learners.

There are also initiatives set up to address growing skills gaps in digital and data talent. #BIGIMPACT, led by the UK Bioindustry Association (BIA), is focused on closing the biotech industry's digital skills gap by encouraging people with digital and data-driven skills to join the sector. Launched in 2023, the campaign's dedicated website and social media accounts highlight potential career pathways in biotech and life sciences, spotlight personal career stories, and list current job vacancies and industrial placements [15].

The International Society for cell and Gene Therapy (ISCT) Institute of Training and Development also delivers globally accredited, CGT translation-focused courses designed by leading experts empowering professionals at every career stage with the knowledge and practical experience to lead in the field. These courses continue to fill a gap in the field by providing specialized training in Laboratory and Manufacturing Practices, Clinical Cell and Gene Therapy, Regulatory Standards, as well as Leadership and Development.

ADVANCE: A PROMISING MODEL

EATRIS, the European Research Infrastructure for translational medicine, places a central focus on training translational researchers through its TransMed Academy, a learning environment for translational scientists including a wide range of self-paced online courses, live courses, recorded webinars, and more.

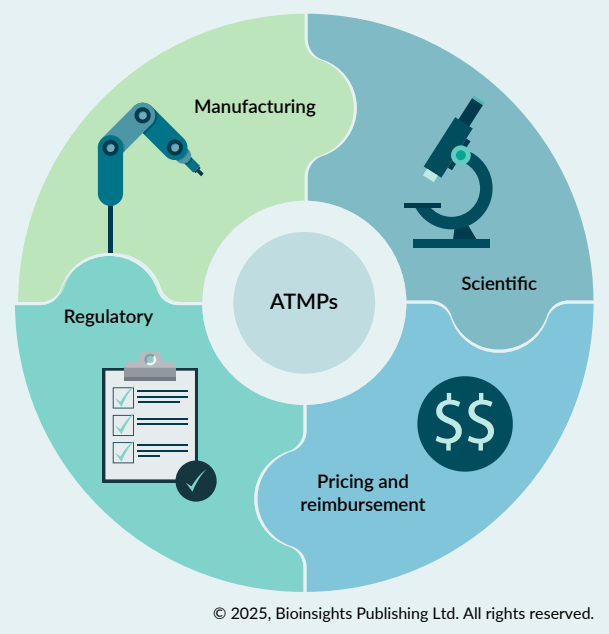
One standout initiative is the ADVANCE program, launched in 2020 with Erasmus+ funding [14]. ADVANCE offers the early career scientist an overview of the four main key areas in ATMP development including scientific, manufacturing, regulatory and pricing and reimbursement (Figure 1). The aim is to entice early career scientists into maybe one pillar of ATMP development, by offering them an overview of each key area. ADVANCE delivers a blended learning model across Europe to support early-career biomedical professionals. It combines online courses, regulatory webinars (in collaboration with the EMA), and in-person, interdisciplinary training across different European countries.

Since launch, ADVANCE has enrolled over 1,000 students, offering: 7+ hours of online lectures and 20 hours of assignments, 7 webinars per cohort, including sessions with EMA experts, and hands-on courses across different European countries.

This approach is flexible, interdisciplinary, and pan-European, and can serve as a blueprint for other training investments. To accompany the online course, 7 in-person events using this format have now taken

FIGURE 1

ADVANCE aims to entice early career scientists in the field of ATMPs through offering training across the entire development pipeline.



place across different regions including Italy, Slovenia, Netherlands, and Belgium, offering this course to local students and the international community. The students at these courses come from all sectors in ATMP development including future scientific leaders, regulators, manufacturers, and economists and Health Technology Assessment (HTA) experts. Enticing early career scientists into this exciting new field across the different areas is core to this program.

WHAT STILL NEEDS FIXING

Despite these efforts, major shortfalls remain:

- Insufficient part-time and online academic qualifications for those already in the workforce
- Lack of targeted funding to support development and delivery of scalable, impactful education and training offers
- Long-term, supported cross sector collaborative effort to address the sector skill needs
- Institutional hesitancy due to uncertain financial returns on modular or part-time training programs

Again, the genomics education model offers inspiration. With dedicated funding and national policy support, the UK embedded genomics training into NHS clinical pathways [8,16]. A similar approach could catalyze growth in ATMP-readiness.

WHAT WOULD A FUTURE-PROOF SKILLS ECOSYSTEM LOOK LIKE?

We need a radical rethink of how we approach workforce development for ATMPs. The following recommendations are key:

- Modular micro-credentials: bite-sized, stackable training units that support flexible, on-demand skill acquisition [2,4]
- Professional/transferable skills (often referred as soft skills) integration: leadership, adaptability, and cross-disciplinary communication must be embedded in all training models [8,17,18]
- Cross-sector secondments: rotational placements can help professionals develop translational fluency [2,18]
- Collaboration between academia, industry, and government to co-design training at all levels that meets real-world needs [18]
- Automation-ready curricula: training must keep pace with MES, AI, and digital twin technologies [5,14]
- Strategic use of AI in education and training development (to speed up translation of content to reach geographically spread target groups; facilitate adaptation of content to new audiences and content, support learners etc.)
- Inclusive, international pipelines: the workforce must be global, mobile, and representative [4,7]
- Long-term political and financial commitment ensuring sustainability, regional equity, and the ability to support innovation from bench to bedside [18]
- Awareness, visibility, and accessibility of CGT careers among school-age students through targeted outreach and engagement, and CGT opportunities at universities by integrating sector-specific content into STEM curricula and promoting internships and placements

CONCLUSION: PEOPLE FIRST, OR PROGRESS ON HOLD

CGTs are no longer speculative, they are here, they are working, and they are changing lives. There are 32 gene therapies now approved globally for clinical use, including treatments for: cancer (e.g., CAR-T therapies like Kymriah® and Yescarta®), genetic disorders (e.g., Zolgensma® for spinal muscular atrophy, Luxturna® for inherited blindness), blood disorders (e.g., Roctavian® for hemophilia A, Casgevy® for sickle cell disease) Over 4,000 gene, cell, and RNA therapies are currently in development worldwide. Of these, 2,042 are gene therapies in various stages from preclinical to pre-registration [19]. Around 1,400 are in preclinical phases alone [20].

But their long-term success hinges not just on scientific brilliance or regulatory clarity, but on people. Without a robust, flexible, coordinated, and inclusive workforce strategy, the promise of ATMP innovation moving safely and efficiently from lab to patient will remain just that: a promise.

We must stop treating workforce development as an afterthought. Instead, it must be seen for what it truly is, core infrastructure for medical innovation. Training initiatives like ADVANCE, ATSTN, IHfGT are foundational. But to truly scale up advanced therapies and meet the needs of patients worldwide, we must double down on investment in modular learning, flexible delivery, and strategic funding. We need to also adapt to a culture of lifelong learning and upskilling to align with the fast pace and needs of the sector. Current skills for today may need to be updated for tomorrow. By not addressing this challenge effectively, the lack of opportunities for workers to cross-train or gain specialized skills could undermine the field's long-term resilience and impact the clear potential of these therapies that offer therapeutic solutions where none currently exist, even curative. We know the challenges, now let us address them together.

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

Scale-up and scale-out of extracellular vesicle production utilizing industrial grade bioreactors

Justice Ene, Falak Syed, Jingjiao Guan, and Yan Li

VIEWPOINT

"As research advances and more discoveries emerge, cell-free extracellular vesicle-based therapies are moving steadily closer to clinical reality."

Human stem cell-derived extracellular vesicles (EVs) play critical roles in cell-cell communication and have been shown to have therapeutic effects, ranging from promoting wound healing to reducing inflammation and protecting against neurodegeneration. The major hurdle of EV translation is the development of a process to increase EV production to a scale at which clinical trials are feasible. Industrial scale up utilizing bioreactors are being investigated to increase EV production while maintaining therapeutic efficacy. Bioreactors introduce a dynamic aspect to EV generation that needs to ascertain the effects of shear rate on EV biogenesis and quality. This viewpoint addresses some insights and discoveries on the forefront of EV scaleup.

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Extracellular vesicles (EVs) are phospholipid bilayer-bound nanoparticles secreted by virtually every cell type and play critical roles in cell-to-cell communication. EVs are classified into three types based on size: apoptotic bodies (1,000–5,000 nm), microvesicles (200–1000 nm), and exosomes (30–200 nm) [1,2]. A key subpopulation of EVs, exosomes, are formed via endosomal sorting complex required for transport (ESCRT)-dependent and -independent pathways [1]. Common positive markers present in exosomes include Alix, TSG101, CD81, and CD63 etc. [2]. The bioactive cargoes within human stem cell-derived EVs, including proteins, nucleic acids, lipids, and growth factors determined by multi-omics analysis, have been shown to possess therapeutic effects, ranging from promoting wound healing to reducing inflammation and protecting against neurodegeneration [3–7]. Although synthetic nanoparticles can be used to load similar cargos, the encapsulation process may compromise the bioactivity of certain therapeutics, such as proteins [8]. In addition, endowing synthetic nanoparticles with precise targeting capabilities remains challenging, and their use is often complicated by the accumulation of foreign materials within the body.

Compared with synthetic nanoparticles for drug delivery, the cellular origin of EVs confers advantages such as intrinsic biodegradability and a reduced risk of immune rejection. Also, owing to their natural role in cellular communication, EVs exhibit tissue-specific homing capabilities, making them promising candidates for targeted therapeutic delivery, including challenging sites such as the central nervous system due to the ability to cross blood–brain barrier [9]. Another notable advantage of EVs in drug delivery is their prolonged circulation time following administration into the bloodstream [10]. In addition, EVs can serve as a substitute for direct stem cell transplantation therapy, offering a less invasive mode of administration while reducing complications associated with cell death, tumorigenicity, and immune rejection. EVs can also be used as non-viral carriers of various types of genetic cargoes for gene therapy applications [11].

A critical obstacle to deploying EVs for treating brain-related diseases is scaling production to meet clinical demand. A 50-study preclinical survey reports a median EV dose of 2.75 mg EV protein per kg of body weight per administration [12]. To meet these clinical requirements, further research is needed to increase EV generation, enrichment, and establish novel scalable production methods [13,14]. Of the two potential strategies to increase production: scale out (increasing the number of active cultures) versus scale up (utilizingng bioreactor cultures), scale out is the least feasible in a long-term perspective from an industrial standpoint. In addition, the downstream large scale EV isolation process also needs to be integrated with the large-scale EV production process. The most feasible scale up process for EV isolation is the tangential flow filtration (TFF) followed by size exclusion chromatography (SEC).

Dynamic 3D culture systems that incorporate bioreactors and mimic physiological flow conditions appear to be the most promising approach for industrial-scale EV production. Currently, the most commonly used bioreactors are rotating-wall and stirred-tank (spinner flask) systems [15–19]. However, these systems have inherent limitations. For example, stirred-tank (spinner flask) reactors generate high shear stress due to horizontal rotation, creating regions with variable shear rates that contribute to both batch-to-batch variability and differences across individual reactor cultures [17,18]. Rotating wall bioreactors also require high rotational speeds to achieve sufficient agitation, which can result in elevated shear forces, and the scalability is limited by the economic costs

of operating large reactors under such conditions [15,16].

Research on novel bioreactor systems such as the vertical wheel bioreactor (VWBR) has shown promise in overcoming the limitations of conventional bioreactors. The vertically rotating wheel occupies more than 80% of the U-shaped bottom volume, generating both radial and axial flow that ensures homogeneous shear rates throughout the culture [20,21]. This unique design allows for efficient mixing at shear levels up to tenfold lower than conventional stirred-tank reactors (and thus reducing the population of ectosomes in the EVs) and has been demonstrated to be scalable to volumes of 500 L [20,21]. The correlation of metabolic status of the parent cells with the EV cargo and the establishment of online monitoring process control may be required for scale up of the EV production from human stem cells.

Recent studies have shown that VWBR can scale up EV production while preserving quality, indicated by the therapeutic cargo profiles. Specifically, human stem cell-derived blood vessel organoids cultured in VWBR, with or without microcarriers, produced significantly more EVs than those grown in 6-well plates [9]. Notably, microcarrier-based VWBR cultures generated nearly fivefold more EVs per million seeded cells than 6-well plates. The EVs retained comparable size and total lipid content, with some differences in lipid chain length and unsaturation that might influence their interactions with target cells. EVs produced from the VWBR cultures also demonstrated therapeutic efficacy, matching or exceeding that of static culture EVs, by reducing oxidative stress and enhancing proliferation in a D-galactose-induced senescence model. Taken together, these findings indicate that VWBR cultures can outperform static methods not only in yield but also in therapeutic potential.

In conclusion, human stem cell-derived EVs hold significant promise as a non-invasive, biocompatible therapeutic, with many avenues remaining to be explored. The greatest hurdle is producing EVs at quantities sufficient to enable clinical experimentation. While scale-out methods are possible, scale-up approaches using bioreactors appear to be a far more realistic path forward. The use of bioreactors introduces additional challenges, such as shear-induced effects on cargo loading and EV membrane composition; however, their ability to substantially increase EV yield is undeniable. As research advances and more discoveries emerge, cell-free EV-based therapies are moving steadily closer to clinical reality.

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BIOGRAPHIES

Justice Ene is currently a doctoral candidate in the Department of Chemical and Biomedical Engineering, FAMU-FSU College of Engineering, at Florida State University. His research focuses on extracellular vesicles therapeutics, understanding how cargo, lipid membrane composition, microenvironment and differentiation effects alleviation of neurodegenerative diseases.

Falak Syed is currently a PhD student in the Department of Chemical and Biomedical Engineering, FAMU-FSU College of Engineering, at Florida State University. Her research focuses on extracellular vesicle (EV) therapeutics, and induced pluripotent stem cell (iPSC)-derived neural organoids, particularly on their assembly, disease modeling, and EV production.

Jingjiao Guan is an Associate Professor of Chemical and Biomedical Engineering at the FAMU-FSIU College of Engineering, Florida State University. For more than two decades he has fused biomaterials science, nanotechnology, and cellular bioengineering to create micro- and nano-scale devices that illuminate and control complex biological processes. Dr. Guan earned his Bachelor of Engineering (1995) and Master of Engineering (1998) in Metal and Materials Physics from the University of Science and Technology Beijing, China then advanced to The Ohio State University, Columbus, OH, USA where he received a Master of Science in Biophysics (2003) and a Doctor of Philosophy in Biomedical Engineering (2005). He remained at The Ohio State University for a National Science Foundation-funded postdoctoral fellowship in nanoscale science and engineering. Since joining Florida State University in 2008, his laboratory has pioneered micro- and nano-scale platforms for biosensing, drug delivery, and fundamental discovery. Recent breakthroughs include engineered microparticles and a predictive mathematical model that together provide unprecedented insight into phagosome biology. Continuously supported by the National Science Foundation, the National Institutes of Health, and the Florida Department of Health, Dr Guan has authored more than 50 peer-reviewed articles in journals such as the Proceedings of the *National Academy of Sciences*, *Advanced Materials*, *Biomaterials*, *ACS Applied Materials & Interfaces*, and *Acta Biomaterialia*. His long-term vision is to integrate biomaterials engineering with cellular engineering to deliver transformative diagnostic and therapeutic solutions for cancer, neurodegenerative disease, and regenerative medicine.

Yan Li is currently a Professor in the Department of Chemical and Biomedical Engineering, FAMU-FSU College of Engineering, at Florida State University. She has more than 20 years of experience in industry and academy on pluripotent stem cell research and technology development. Dr Li received her BS degree in Chemical Engineering from Tsinghua University, China and her PhD in Chemical Engineering from The Ohio State University, Columbus, OH, USA. At The Ohio State University, Dr Li received numerous awards including Presidential Fellowship and Graduate Student Alumni Research Award. From 2002 to 2011, Dr Li worked at Geron Corporation. In 2011, Li joined the Department of Chemical and Biomedical Engineering at FAMU-FSU College of Engineering, Florida State University. She is a Visiting Professor at Mayo Clinic during 2025–2026. Dr Li has received numerous grants from National Science Foundation (NSF), National Institutes of Health (NIH), and Florida Department of Health (FLDOH). Institutionally, Dr Li received Florida State University (FSU) Developing Scholar award, Innovator awards, Outstanding Research Accomplishment award of FAMU-FSU College of Engineering, Outstanding Research Award of the Department, and completed the FSU Emerging Leaders Program. Nationally, Dr Li received the prestigious NSF CAREER award, Cell Culture Engineering XV Organizer award, and Exceptional Speaker award from the Society for Biological Engineering. She is a Fellow of Executive Leadership in Academic Technology, Engineering and Science (ELATES) and a Fellow of American Institute for Medical and Biological Engineering (AIMBE). Dr Li is a panelist for multiple NSF and AIMBE programs, American Heart Association, numerous NIH study sections as an Ad Hoc and a standard member, and a reviewer for more than 60 journals. Dr Li has more than 140 peer-reviewed publications, 13 patents or patent applications, and >200 presentations primarily from Florida State University.

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

AAV production and purification: key steps from design to GMP readiness

Goutham Kumar Ganjam, Emily Jackson-Holmes, Florian Leseigneur, Sravanthi Pasupuleti, Marco Wachowius, Helena Martins, and Ayuso Eduard

AAV manufacturing presents challenges across both upstream and downstream processes, each requiring optimization to facilitate robust and scalable production. The following studies focus on AAV production workflow and how different steps within the upstream and downstream processes were optimized. Namely, cell expansion, plasmid transfection, and vector production in the upstream process, and purification and analytics in the downstream process. Case studies highlight how critical raw materials and process parameters were chosen to enable productivity and scalability as well as how to meet the quality target product profile of the AAV produced.

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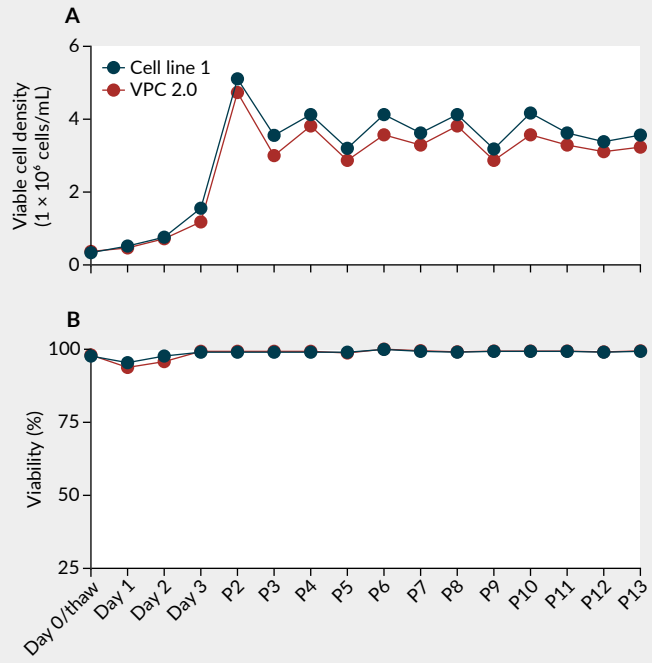
INTRODUCTION: AN AAV PLATFORM PRODUCTION PROCESS

The production process for AAV viral vectors is as follows: the first step of the upstream process is cell culture. Suspension-based cell lines are used for viral vector manufacturing and GMP-compatible single-use consumables or materials. Following cell expansion, the next step involves inoculating the bioreactors with the expanded cell culture. This is followed by triple plasmid transfection for viral vector production. After a 72 h incubation period, the virus is harvested by chemical lysis and the resulting crude lysate is clarified using depth filtration. The clarified harvest then undergoes downstream process purification by ultrafiltration/diafiltration (UF/DF) and is subsequently loaded onto the affinity chromatography column. Depending on the end goal, the affinity captured material can undergo scalable anion exchange chromatography or ultra-density gradient ultracentrifugation steps for full capsid enrichment. The process concludes with final buffer exchange and concentration for final formulation.

In viral vector manufacturing, there are several factors that can be optimized during the upstream process including plasmid ratios, total DNA amount, cell lines, media, and transfection reagents. These

FIGURE 1

Comparison of cell density (A), viability (B), and cell distribution (C) between VPC 2.0 and a competitor cell line.



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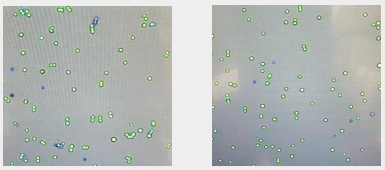


Parameters	Cell line 1	VPC 2.0
After thawing VCD and viability	2.5×10^5 with 97.3%	3.0×10^5 with 98.3%
Viaible cell density (cells/mL) lowest	2.5×10^5	3.0×10^5
Viaible cell density (cells/mL) highest	5.2×10^6	4.8×10^6
Viability (min)	95.8%	93.7%
Viability (max)	99.4%	99.6%

parameters can be fine-tuned using techniques such as DoE using QbD principles to improve yield and quality, which help ensure a smooth and efficient scale up to larger bioreactor volumes and later, to clean room manufacturing.

Comparison of cell density and viability between Viral Production Cells 2.0 and a comparator cell line

A comparison of cell density and viability between Gibco™ Viral Production Cells 2.0 (VPC 2.0) and a competitor cell line (cell line 1) was conducted. Both are suspension HEK-293 cell lines. The results showed comparable cell growth kinetics and population doubling time (Figure 1). Furthermore, microscope images showed that VPC 2.0 cells exhibited greater monodispersity (i.e. uniform single cell distribution) compared to cell line 1. Monodispersity is important to achieve consistent and reliable transfection, which is crucial for efficient and scalable AAV manufacturing.

The impact of different helper plasmids on AAV production

This study compared AAV9 production between the VPC 2.0 cell line and cell line 1 using three different sets of *rep/cap* and helper plasmids (A, B and C in Figure 2). The same gene-of-interest (GOI) was used throughout. Transfection was carried out at a recommended viable cell density. Following 72 h post-transfection, cells were chemically lysed, and AAV9 was harvested through two clarification streams.

Plasmid set B yielded the highest viral vector genome titers in both cell lines. Overall, the VPC 2.0 cell line suspension

Explore more on this topic by reading these supplementary brochures:
Gibco AAV-MAX AAV Production System brochure and **Production of AAV at the 1,000 L scale in the DynaDrive Single-Use Bioreactor using the CTS AAV-MAX production system**

showed slightly higher titers at the clarified harvest stage (quantified by qPCR) compared to cell line 1, highlighting its improved productivity.

Assessment of full capsids in clarified harvest by mass photometry

Mass photometry was used to determine full-to-empty capsid ratios of AAV particles from clarified harvest samples. AAV particles were captured using Dynabeads™ CaptureSelect™ AAVX Magnetic Beads with a small sample up to 1 mL. This was conducted on material generated from both cell lines prior to downstream purification. The results showed that VPC 2.0 cell line had an almost 3-fold higher percentage of full capsids compared to cell line 1, indicating a significantly improved AAV encapsulation rate (Figure 3).

Comparison of purification using Dynabeads CaptureSelect AAVX Magnetic Beads vs POROS™ CaptureSelect™ AAVX Affinity Resin

A study was conducted to evaluate the effectiveness of capture by Dynabeads CaptureSelect AAVX Magnetic Beads compared to POROS CaptureSelect AAVX Affinity Resin in a large-scale production setting. As shown in Figure 4, both capture approaches yielded comparable percentages of full capsids analyzed from clarified harvest. These results were consistent with small-scale models and confirm that the magnetic bead-based capture performs on par with traditional affinity chromatography. This means that either approach can be applied based on the specific requirements of the project, while still benefiting from the high encapsulation efficiency of VPC cells.

UPSTREAM PROCESS OPTIMIZATION

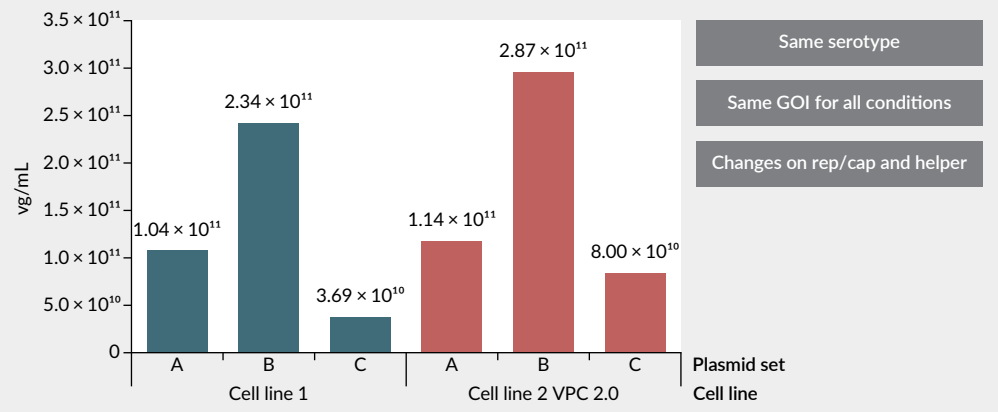
Feasibility study with two transfection kits

As part of AAV upstream process optimization, the transfection step was studied by comparing the effectiveness of two different transfection reagents on viral titer, full-to-empty capsid ratio, and AAV productivity. The transfection reagents that were compared were the Gibco™ AAV-MAX Transfection Kit and a comparator (Kit B). The VPC 2.0 cell line was used in both cases.

The results from the clarified harvest show that the AAV-MAX Transfection Kit

FIGURE 2

Comparison of viral titer between VPC 2.0 cell line and cell line 1 using three different sets of *rep/cap* and helper plasmids (A, B, and C).



produced a higher viral titer (Figure 5A) as well as a greater percentage of full capsids (20.5% vs 13.8%; Figure 5B) compared to Kit B.

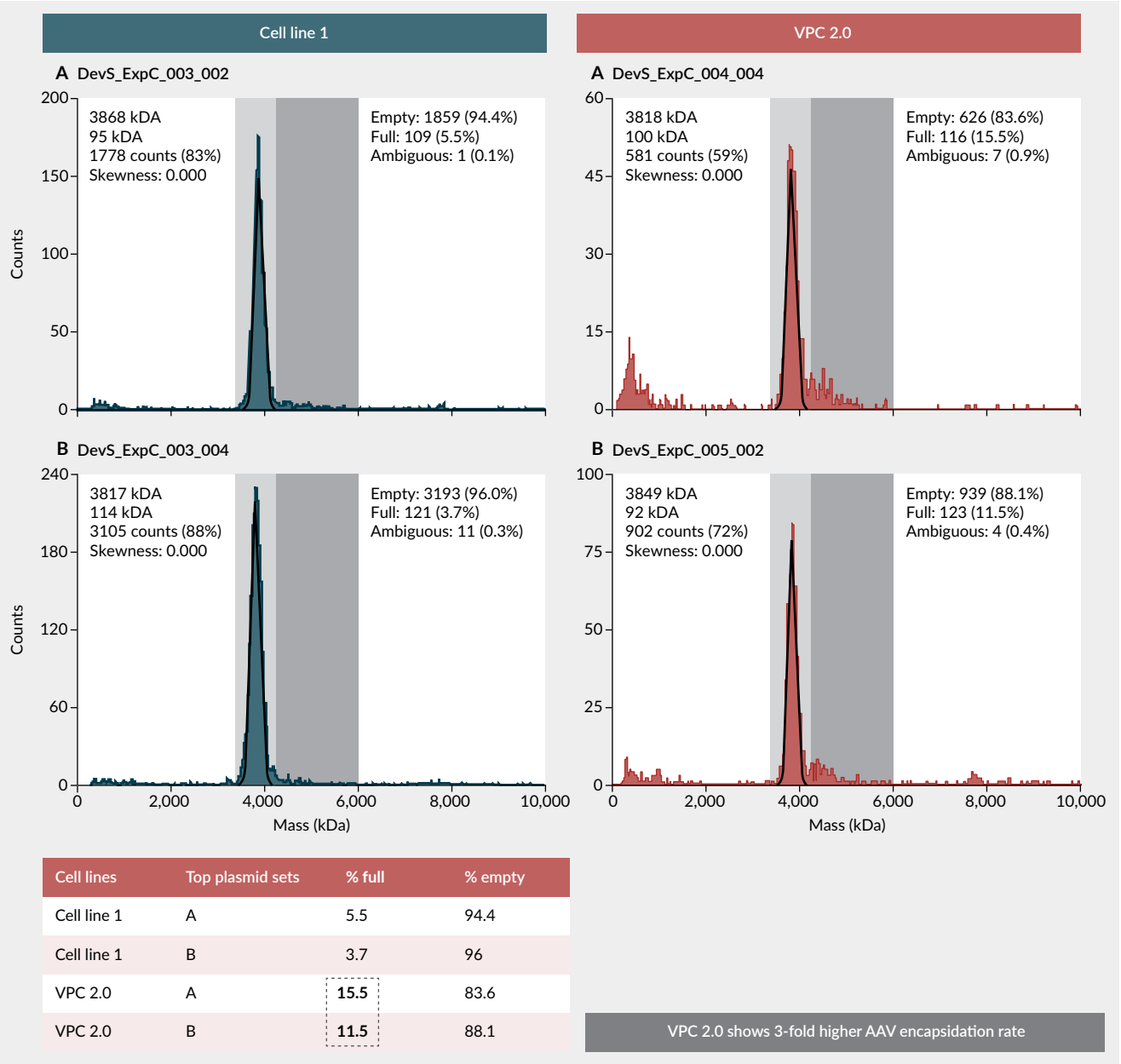
Upstream bioreactor scale-up

Insights learned from upstream optimization and the identification of critical process parameters were used as a foundation to scale up to 2 L bioreactors.

Regardless of whether the process is at 2 L or 50 L scale, the process remains the same: seeding, expansion, inoculation, transfection, and harvest clarification. Gas sparging profiles and other critical process parameters are measured during

FIGURE 3

Full-to-empty capsid ratio following magnetic AAVX capture as determined by mass photometry for VPC 2.0 cell line and cell line 1.



Cell lines	Top plasmid sets	% full	% empty
Cell line 1	A	5.5	94.4
Cell line 1	B	3.7	96
VPC 2.0	A	**15.5**	83.6
VPC 2.0	B	**11.5**	88.1

VPC 2.0 shows 3-fold higher AAV encapsidation rate

production. These insights enable conditions to be fine-tuned and validated during pilot runs at both 2 L and 50 L scales. This approach generates sufficient clarified material for downstream process optimization and confirms process robustness at different scales.

Suspension VPC 2.0 cell growth kinetics in stirred-tank bioreactors

The behavior of VPC 2.0 cells was studied at different stages within the bioreactor, from N-1 stage until final harvest. Microscope images showed most VPC 2.0 cells to be monodispersed. The cells grew well in the bioreactor and reached almost 5.1 million cells with >95% viability. From Day 3, once optimal seed density was achieved, cells were transfected and then subsequently harvested after 72 h.

AAV scale-up: upstream process from flask to 50 L

The next study analyzed viral genome titer using the AAV-MAX system across different scales. Figure 6 shows the total vector genome at clarified harvest from 0.5 L to 50 L. The results showed a high viral titer at the higher scales. Analysis of vg/ml at

FIGURE 4

Comparison of purification between AAV magnetic beads vs affinity chromatography.

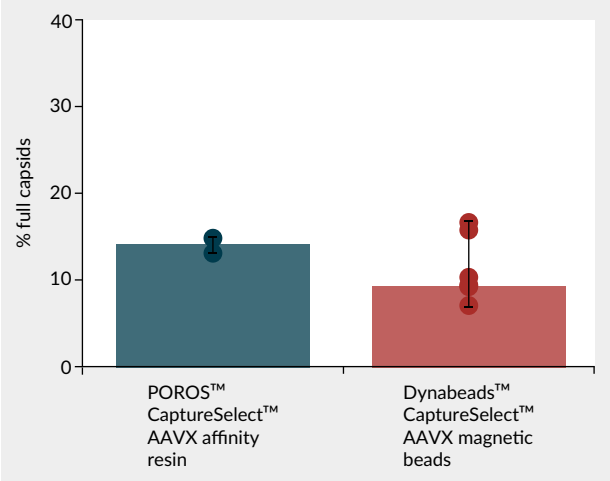


FIGURE 5

Comparison of viral titer (A) and full-to-empty capsid ratio (B) for the AAV-MAX Transfection Kit and Kit B.

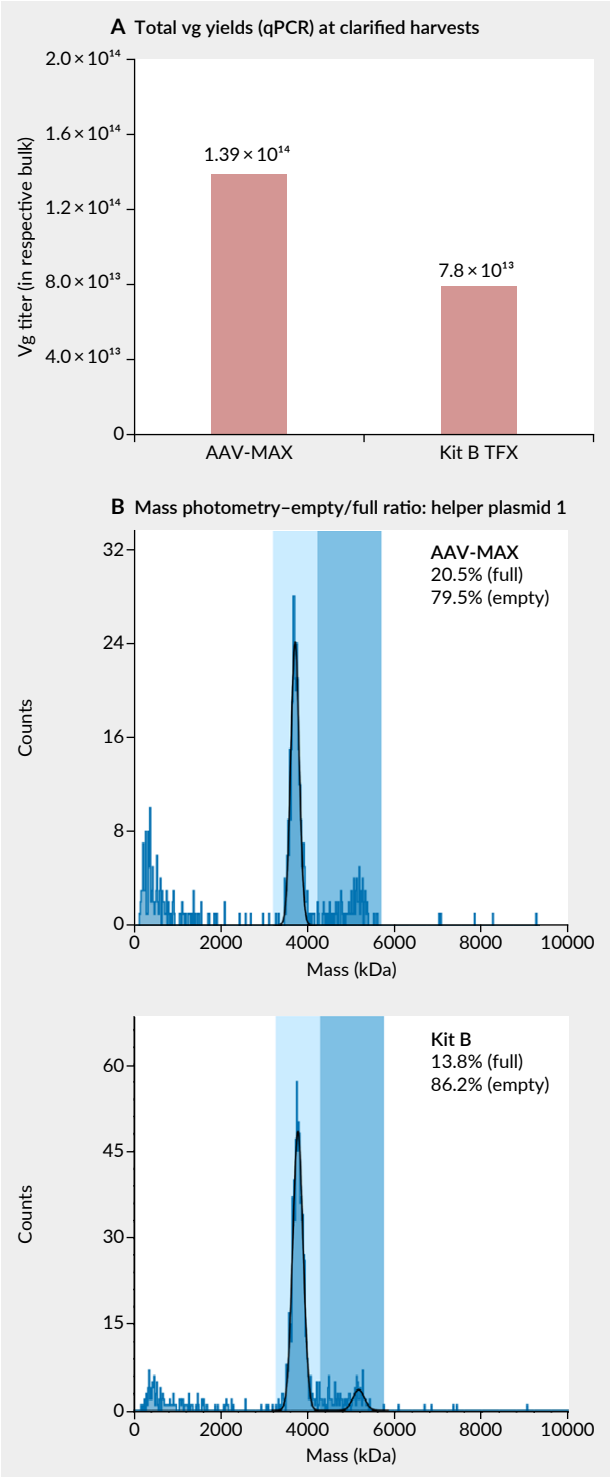


FIGURE 6

Total vector genome (A) and vg/mL (B) at clarified harvest.

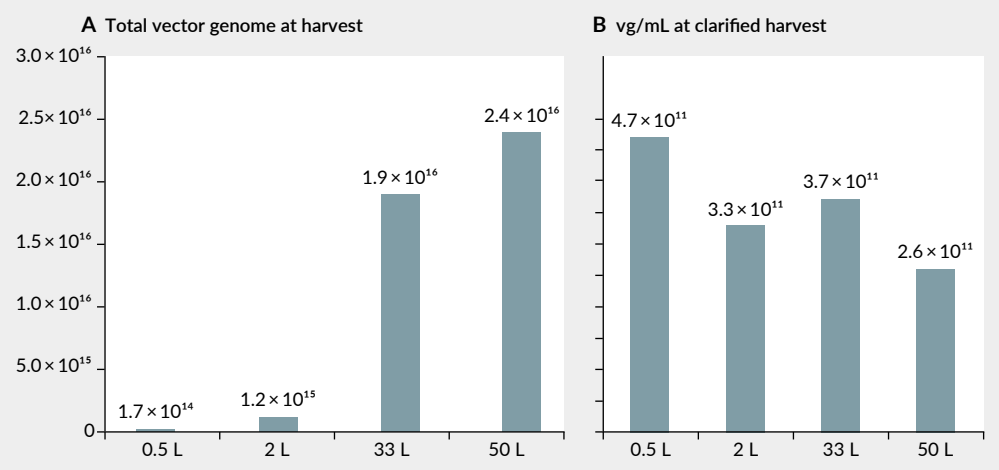
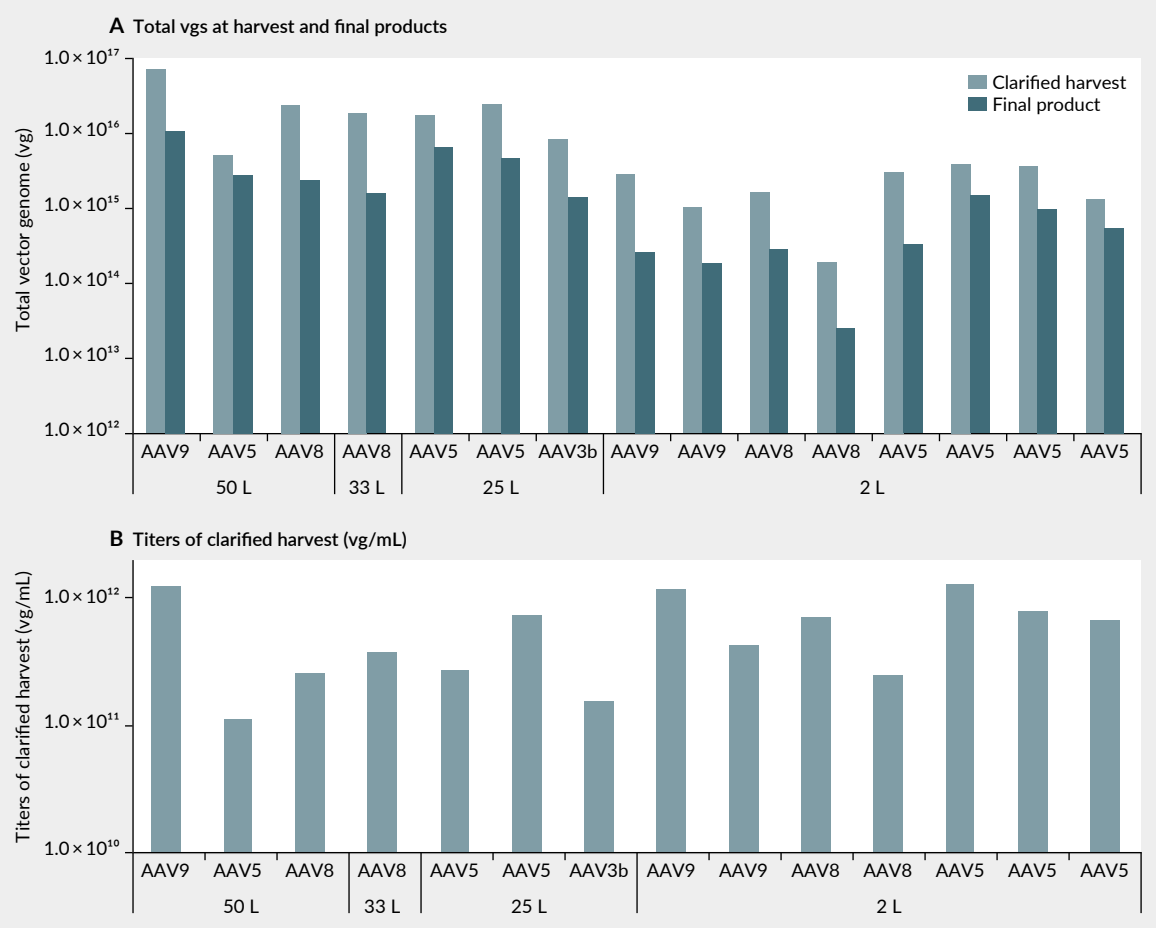


FIGURE 7

Total vector genome yield at clarified harvest and final product (A) Titer of clarified harvest (B).



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clarified harvest showed comparable levels from small to large scales. These results confirmed the process reproducibility and translatability at different scales.

Viral titer in stirred-tank bioreactors

The following results highlight the robustness and flexibility of the upstream and downstream processes across different AAV serotypes and production scales. Figure 7A shows total vector genome yields at both clarified harvest and final product stage. Total vector genome yield was consistently high across the 2 L to 50 L scale range, regardless of serotype. Figure 7B further confirms that clarified harvest titers were maintained across different bioreactor volumes, demonstrating process scalability and serotype adaptability.

In conclusion, by using the AAV-MAX system this platform is able to support high AAV viral titers with reproducible performance across different AAV serotypes and production scales. As such, this platform can be used in early-stage R&D as well as larger scale, GMP-compliant clinical manufacturing.

DOWNSTREAM PROCESS OPTIMIZATION

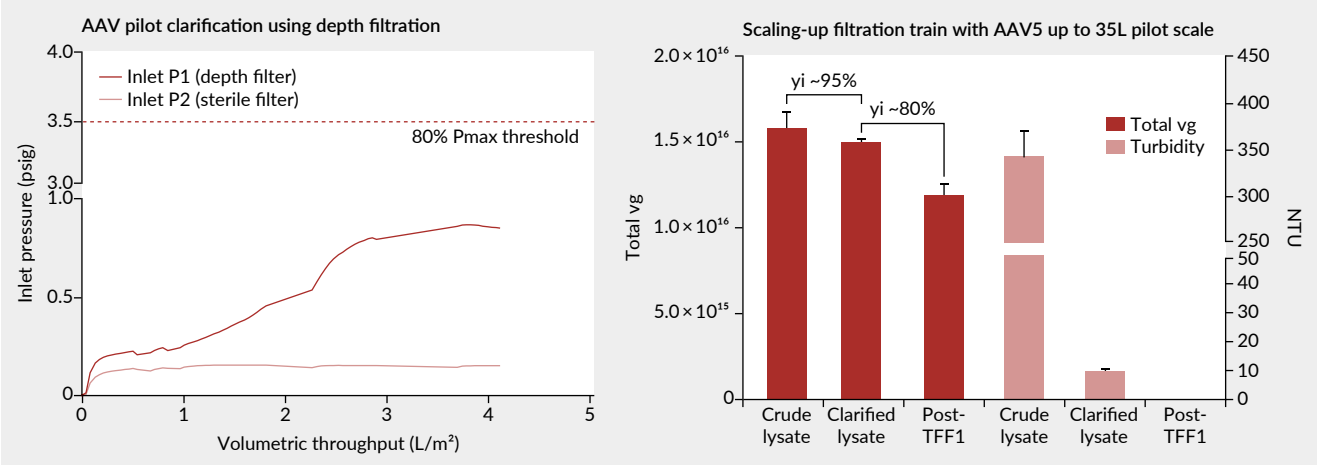
Challenges and solutions for AAV downstream processing

Developing a robust AAV downstream process involves navigating several challenges. The main challenge is achieving a balance between high purity and high yield while ensuring efficient throughput, all while keeping costs as low as possible.

Platform process design commonly involves single-use stirred- tank bioreactors, other single-use technologies, and a suspension cell line. The platform should also encompass a wide array of analytical capabilities to characterize and quantify product-related metrics and impurities. The incorporation of scalable technologies is another necessary part of platform design. Additional challenges during downstream development include establishing the most suitable platforms during early scale-down studies. These platforms help characterization and optimization of a baseline process, which enables the transition to larger scale platforms and eventually, to GMP-compliant manufacturing.

FIGURE 8

Filter capacity curve during AAV pilot-scale clarification using depth filtration (A); AAV5 vector genome recovery following each fitration step of the filtration train (B).



In the studies that follow, various techniques and equipment were employed such as single-use bioreactors, scalable filtration within the filtration train, and a combination of orthogonal separation techniques, such as tangential

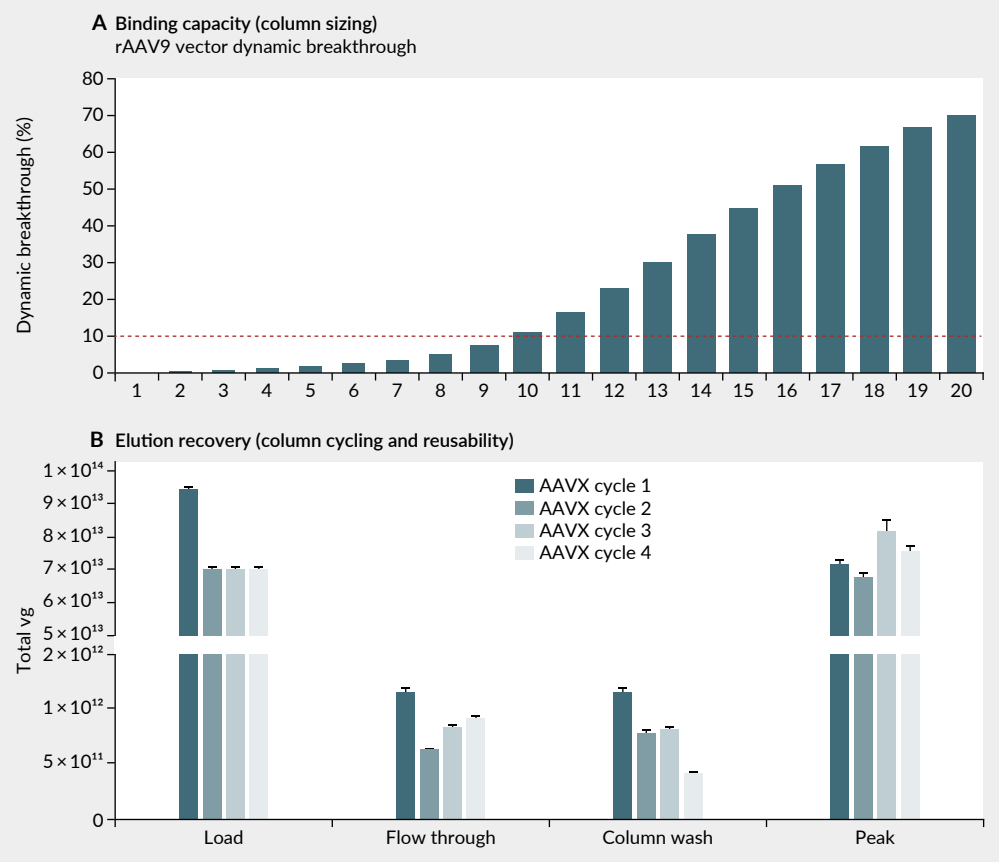
TABLE 1

Settings of key process parameters used and outcome of resulting metrics.

Process parameters	Settings
Filter sizing/ratio	4:2:1
Filter pore size rating (µm)	5–0.2
Filter media chemistry	Regenerated cellulose
Target feed flux, J_f (LMH)	50
Key metrics	**Outputs**
Overall vg yield	75%
Turbidity reduction	34X
Filtering capacity (% Pmax)	20%

FIGURE 9

Impact of column sizing on binding capacity of the affinity resin using rAAV9 vector (A); impact of column cycling and reusability on elution recovery over four cycles (B).



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flow filtration (TFF) and chromatography-based techniques.

Scaling-up AAV filtration train

The initial study focused on scaling up the AAV filtration train, with insights gathered from a 35 L pilot production. The process involved utilizing soluble detergents and a complex matrix to purify crude lysate from high-density host cell impurities.

A preliminary scale-down clarification study helped determine filter selections, filter sizing, and key metrics such as vector mass balance, filter capacity, and turbidity reduction. The filter capacity curve shows that the process was able to operate well below Pmax (Figure 8A). Analysis by PCR shows the high vector genome recovery after each filtration train step (i.e. harvest, clarification, and pilot TFF (Figure 8B). Turbidity reduction is >30-fold. The main consideration during scale-up was to adjust the operating conditions in a way that achieved an optimal balance between throughput and capacity while also applying a margin of safety (Table 1).

High-capacity pan-affinity resin for AAV capsids

In the next study, POROS CaptureSelect AAVX affinity chromatography was used to selectively bind and elute AAV9 serotype. The two key metrics analyzed for process optimization were column sizing and column cycling (Figure 9). A pan-affinity resin was used. The data presented are derived from inverted terminal repeat (ITR)-based vector genome copy quantification.

The breakthrough curve indicates resin demand, which is estimated based

TABLE 2

Estimation of column volume using productivity and encapsidation rate

X/Y (%)	5.0×10^{10}	7.5×10^{10}	1.0×10^{11}	2.5×10^{11}	5.0×10^{11}
1	26.7	40.0	53.3	113.3	266.7
5	5.3	8.0	10.7	26.7	53.3
10	2.7	4.0	5.3	13.3	26.7
15	1.8	2.7	3.6	8.9	17.8
20	1.3	2.0	2.7	6.7	13.3
25	1.1	1.6	2.1	5.3	10.7
30	0.9	1.3	1.8	4.4	8.9

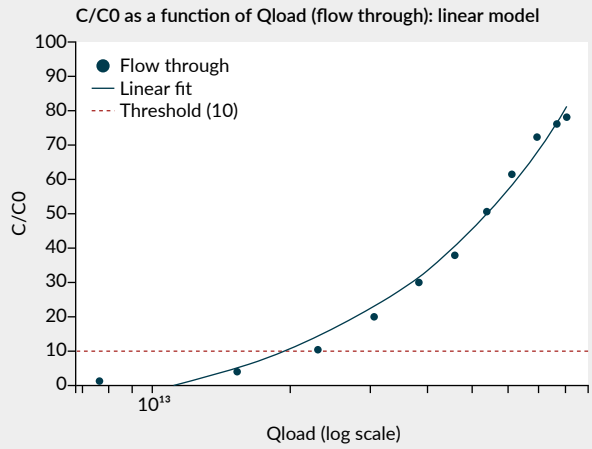
TABLE 3

Binding–elution performance between the vector capsid and protein ligand over four cycles.

Cycle	Loading density (vg/mL resin)	% binding (vg)	% elution (vg)
AAVX Cycle 1	1.34×10^{14}	98	76
AAVX Cycle 2	9.95×10^{13}	95	96
AAVX Cycle 3	9.95×10^{13}	98	111
AAVX Cycle 4	9.95×10^{13}	98	103

FIGURE 10

Breakthrough curves, using linear and polynomial models, of rAAV9 vector on POROS CaptureSelect AAVX affinity resin.



Model	R2 score	MSE
Linear	0.988	9.186
Polynomial	0.989	8.489

	Cycle 1	Cycle 2
AAV9 cp/mL	6.5×10^{14}	5.0×10^{14}

on binding capacity studies. For example, if the upstream productivity (X-axis) is 1×10^{11} vg/ml and the encapsidation rate is 10%, then the required column volume is calculated to be 5.3 mL (Table 2).

Another factor that is essential to optimizing the binding–elution process is establishing reproducible binding–elution performance between the vector capsid and protein ligand. In this study, high-binding performance was observed over four cycles. There was a two-load difference in the flow through with minimal vector breakthrough. Vector genome elution recovery was consistent (Table 3).

Measurement of dynamic binding capacity using rAAV9 vector

A dynamic binding capacity study was performed to assess how much viral vector can bind to a resin on an affinity-based platform. Figure 10 illustrates breakthrough curves of the rAAV9 vector on POROS CaptureSelect AAVX affinity resin, using two different breakthrough calculation methods. Binding capacity was assessed over time by combining both column sizing and column cycling. The results showed a continuous decay in binding capacity through different mechanisms such as ligand leakage or irreversible binding interaction. Understanding the resin's binding capacity is essential before proceeding to scale up, as this gives an insight into the quality of starting material from the upstream process and the capacity of ligand regeneration.

Handling multiple AAV serotypes and engineered capsids

A further study was performed to explore the performance of POROS CaptureSelect AAVX affinity resin for different AAV serotypes.

During early process development, a preliminary purification screening study was conducted to assess the binding–elution behaviors of two different serotypes designated AAV-a and AAV-b. To assess vector recovery and impurity clearance, the following analytical techniques were employed: PCR for vector genome quantification, mass balance analysis, UV absorbance chromatograms, and SDS-PAGE to assess preliminary protein impurity

clearance and to check the integrity of viral protein bands.

There were two main challenges with this study; the first was the fact that AAV-a showed strong binding affinity that required optimization. Prior to optimization, AAV-a exhibited desorption of 40%, whereas after optimization, this was enhanced to >90%. The second challenge was the reverse issue - i.e. serotypes that were poor binders which could escape the binding epitope. In these cases, empirical testing was needed to assess if the pan-affinity resin was reliable for use in this platform process. A small-scale study was conducted to compare the binding-elution performance of two different affinity resins on the AAV-b vector. The results showed that the POROS CaptureSelect AAVX resin outperformed the competitor resin across two different batches. Offline analysis with PCR or analytical chromatography confirmed this result.

Affinity capture screening study with rAAV2

To further refine affinity capture process development, a DoE approach was used to systematically identify the design space and the optimal combination of parameters to maximize elution recovery. Among the tested conditions, use of a POROS CaptureSelect AAVX affinity resin resulted in a six-fold difference in vector recovery.

rAAV2 purification at 5 L produced by rocking motion bioreactor

This scale-down study was translated to bench-scale using 5 L rocking motion bioreactors with single-use technology. The AAV2 material produced from suspension cells was scaled up using a packed-bed column.

The separation profile shows a sharp elution peak, with a second residual peak observed during column clean-in-place indicating some minor carryover (Figure 11A). Offline vector genome quantification showed minimal vector breakthrough (<1%) in the flow through, which is a >2-log reduction (Figure 11B). Process performance was measured at >70%, which is consistent with the DoE results achieved with a small-scale study.

AAV pilot purification: demonstration of scale

Following early-stage development, which established parameters relating to binding performance, column sizing, cycling capacity, and optimal desorption conditions, a scale-up study of POROS CaptureSelect AAVX affinity resin was initiated to evaluate linear scalability using a defined set of parameters.

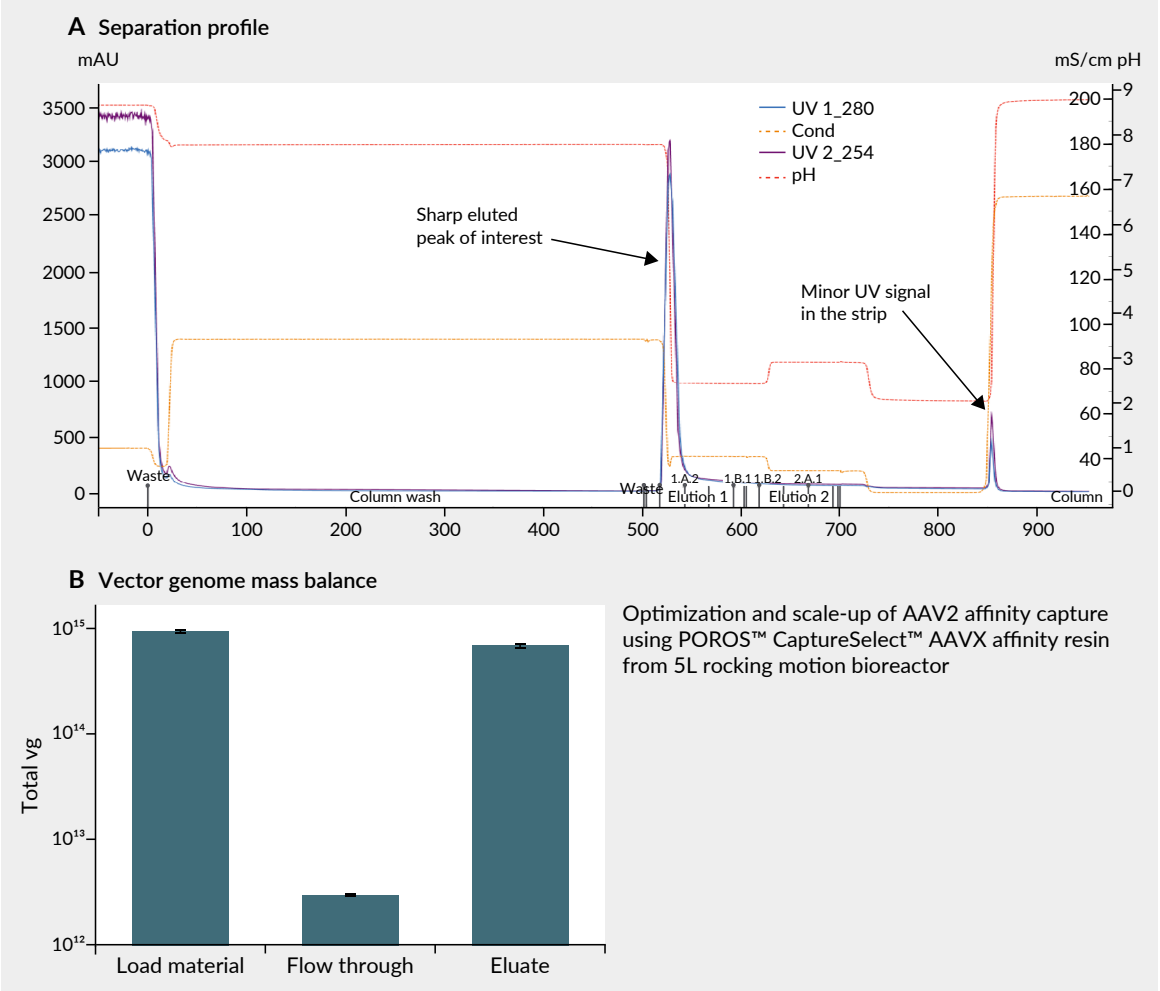
In this study, the downstream process was scaled up to 50 L stirred-tank bioreactors. The AAV affinity resin was resized to approximately 140 mL and a two-cycle approach was applied following TFF. The resulting purification profile showed an elution peak indicating target vector genome. Other peaks showed no vector genome present, as confirmed by PCR. The intermediate vector genome recovery was almost 70% from harvest to post-affinity capture, indicating a high performance from small scale to bench scale.

Process development of a polishing step using anion exchange chromatography

Establishing a scalable downstream process for AAV relies on combining multiple unit operations. One particularly challenging step is polishing. In the next study, anion exchange chromatography was used as the polishing technique. AAV8 was the chosen serotype. DoE was used to identify critical process parameters and map a suitable design space based on process constraints and targets. A trade-off between high yield and high purity was observed.

FIGURE 11

Chromatogram of separation profile of AAV2 during purification (A); vector genome quantification of AAV2 using POROS CaptureSelect AAVX affinity resin from 5 L rocking motion bioreactor (B).



Optimization and scale-up of AAV2 affinity capture using POROS™ CaptureSelect™ AAVX affinity resin from 5L rocking motion bioreactor

A 50 L pilot upstream process study was performed involving the purification of two independent batches. The data indicated a strong translation from the scale-down to the scale-up study. Furthermore, the AAV8 pilot platform achieved vector genome yields between 1×10^{15} and 1×10^{16}, which was sufficient for use in large preclinical animal studies. To proceed with these studies, rapid scale-up was required and was completed in <2 months. One key feature of the platform that enabled efficient scale-up was the pan-affinity ligand, which simplified the capture process.

Pilot AAV manufacturing—QC overview

To conclude on the AAV downstream process, defining the quality target product profile (QTPP) is a crucial step. It involves evaluation of vector genome and capsid titers, vector particle infectivity, and process and product-related impurities such as endotoxin, host cell protein, and host cell DNA. By applying process development for both small-scale and scaled up production, the two pilot AAV batches showed a comparable quality profile in terms of product quantification and impurity profile.

Q&A

Goutham Kumar Ganjam

Q What are some best practices for optimizing transfection conditions (e.g., plasmid ratios, transfection reagents) to maximize AAV production?

GG In order to optimize transfection conditions, several factors must be considered. Firstly, triple plasmid transfection commonly uses a 1:1:1 ratio. By applying QbD and DoE methods, the correct ratio of GOI to its respective helper plasmids can be identified. Notably, different GOIs can present unique challenges, which would in turn require tailored approaches. Other crucial parameters include determining an appropriate cell density and identifying media that are most compatible with the transfection reagent while also avoiding cytotoxicity.

Furthermore, to facilitate seamless scale up, all reagents need to be compatible with both small- and GMP-scale manufacturing. As such, it is important to consider these various parameters from early stages of development in order to maximize AAV production efficiency.

Q What are the critical parameters to monitor during cell culture to enable consistent and high-quality AAV production?

GG The most critical parameter at the cell culture stage is the growth kinetics of the production cell line. Questions that need to be asked at this stage include: what is the viable cell density? What is the viability? What is the population doubling time? What is the most suitable suspension culture media (or should another media be considered)? These factors are particularly important at the point of transfection.

Parameters such as pH of the media and dissolved oxygen are also important both at the shake flask level and larger pilot-scale productions. pH can vary throughout the process and therefore requires frequent monitoring.

Cell health is a critical factor in achieving consistent and high-quality AAV production. It is necessary to consider what metabolic pathways are relevant for the specific viral vector. Glucose consumption, lactate production, and ammonia levels need to be carefully monitored to enable cell health and the achievement of high-quality vector product. A solid understanding of the producer cell line is also essential. Cell morphology is another critical parameter, especially in suspension cultures where aggregation remains one of the biggest challenges. Optimizing the media to maintain cells in a monodispersed state is crucial to transfection efficiency.

Q You tested different *rep/cap* and helper plasmids. Can you comment on how these plasmids differed and what genetic manipulations were performed to increase AAV production?

GG Out of the three helper plasmid sets, plasmid set B included the *rep/cap* plasmid and an adenoviral helper plasmid, which is essential for AAV production. The design of the *rep/cap* plasmid and its promoter selection can influence yield. In addition, adenoviral helper proteins such as E2A, E4, or VA play a crucial role. These can be described as 'first generation' helper plasmids.

In recent years, helper plasmids have been upgraded to give rise to new-generation helper plasmids, which are commercially available from R&D to GMP grades. These plasmids are high quality and harbor additional helper virus elements without compromising safety. For example, some elements of the herpes simplex virus are known to influence viral titer and full-to-empty capsid ratio, thus affecting the quality and yield of the viral vector.

Studies have shown that there are differences in AAV production when using new-generation helper plasmids compared to early generation plasmids. In the above study, plasmid set B contained adenoviral helper elements but also other viral helper elements.

Q You were able to optimize your process up to the 50 L scale. Do you have plans to scale up to larger vessels and if so, do you think the parameters for 50 L will translate to these larger scales?

GG I have heard pioneers in gene therapy saying that if you have a fantastic gene therapy molecule but you can't manufacture it, consider it to be a failed gene therapy. Our approach is to begin with the end in mind. From a very early stage, it is important to optimize process parameters at both small-scale and pilot large-scale models.

It is important to optimize key parameters such as mixing and mass transfer of gases into the media, as well as shear stress, cell viability, and bioreactor design. If the geometry between bioreactors is similar, then scaling up will not require much adjustment of key process parameters. This can give 80–90% confidence in the ability to scale up from 50 L pilot scale to 200 L or 500 L. Yes, scaling beyond 50 L is part of our strategy.

BIOGRAPHY

Goutham Kumar Ganjam is an AAV manufacturing expert specializing in scalable viral vector process development from gene cassette design to 50-L pilot scale. At Siegfried DINAMIQS and DINAQOR, he built robust, GMP-ready upstream platforms for therapeutically relevant indications, translating preclinical workflows into high-yield, scalable production systems.

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Siegfried DINAMIQS is transforming viral vector manufacturing for the next generation of cell and gene therapies. Led by industry veterans Martin Kessler CEO and Eduard Ayuso CTO, whose CGT scientific expertise anchors its innovation, the Swiss-based hub combines Siegfried's global pharmaceutical infrastructure with DINAMIQS's cutting-edge development platform. With its new 2 × 500 L GMP facility in Zurich-Schlieren, Siegfried DINAMIQS is set to deliver scalable, high-quality viral vectors and redefine standards of quality, reliability, and patient impact worldwide (**www.dinamiqs.com**).

Gibco AAV-MAX AAV Production System brochure

Production of AAV at the 1,000 L scale in the DynaDrive Single-Use Bioreactor using the CTS AAV-MAX production system

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

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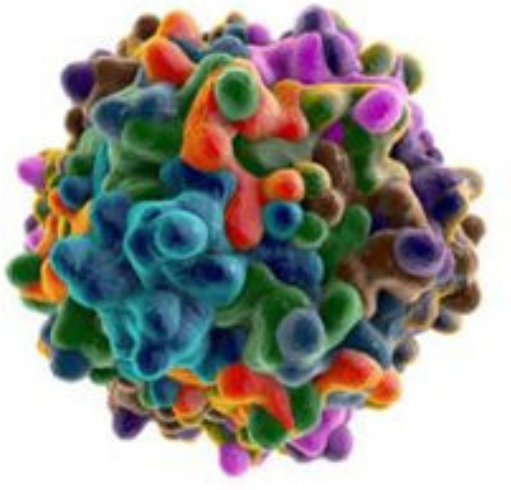

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Overcoming CMC hurdles in TIL therapy: strategic insights from a clinical-stage biotech

Alex Lei and Sabrina Carmichael

With the ever-growing need for precise, personalized, and effective therapeutics, tumor-infiltrating lymphocyte (TIL) therapy offers a promising approach to treating solid tumors by harnessing the patient's own immune cells and addressing challenges such as tumor heterogeneity. This article explores GRIT's pipeline of personalized therapies, and a comprehensive strategy to overcome key CMC challenges in TIL development—including tumor sampling, feeder cell safety, potency assays, and cryopreservation. Clinical outcomes, scalable manufacturing solutions, and future directions for automation and process optimization in TIL-based immunotherapy are also explored.

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FROM LIQUID TO SOLID TUMORS: EXPANDING THE REACH OF T CELL THERAPIES

T cells are at the core of cancer immunotherapy, driving the success behind numerous treatment modalities. For example, PD-1/PD-L1 immunotherapy, which is based on the interaction between tumor cells and T cells, have shown very promising results in late-stage oncology patients. The same mechanism of action is observed with oncolytic viruses, inhibitory cytokines, and targeted regulatory T cells.

Chimeric antigen receptor (CAR) T cell therapies have also been making an impact on patients, with FDA-approved treatments available for several types of liquid tumors. In particular, CD19- and BCMA-targeted CAR T cells have demonstrated strong overall response rates and long-lasting effects. However, this success has not yet translated to solid tumors.

In the liquid tumor space, most antigens are universally present on cancer cells for example, CD19 or BCMA antigens. In contrast, there is no such uniformity for solid tumors; instead, a mixture of antigens is observed. For instance, when CAR T cells target HER2- or EGFR antigens, they may eliminate some of the solid tumor, but cancer cells without these antigens can

survive and proliferate, ultimately leading to unsatisfactory patient outcomes.

Additionally, the tumor microenvironment (TME) inhibits T cell functionality after entering the solid tumor space, posing an additional challenge. T cell exhaustion must also be considered, which has been observed in both liquid and solid tumor settings.

To tackle challenges in treating solid tumors, tumor-infiltrating lymphocyte (TIL) therapy utilizes T cells extracted from a patient's tumor to produce a diverse population of tumor-targeting cells particularly well-suited to address antigen heterogeneity.

UNIQUE ADVANTAGES OF TILs OVER OTHER IMMUNOTHERAPIES

One key difference of TIL therapies compared to CAR T and T cell receptor T (TCR-T) therapies is the cell source: T cells are extracted directly from a tumor, as opposed to peripheral blood mononuclear cells (PBMCs). Within a tumor, a polyclonal T cell population exists to target the various antigens on tumor cells. Expanding this population for TIL therapy preserves their diverse clonality, enabling broad targeting of a heterogenous solid tumor.

Extracting TILs directly from the target tumor is advantageous as this population has already proven to have a balanced expression of chemokine receptors that enables them to penetrate the TME and target the tumor.

Lastly, since T cells are naturally occurring, cytokine release syndrome or off-target toxicity, which can occur with CAR T cell therapies, is typically not observed with TIL therapy.

THE EVOLUTION OF TIL THERAPIES

TIL therapy was first developed in 1988 when Steven Rosenberg from the National Cancer Institute (NCI) first reported the use of TILs in the treatment of melanoma [1]. However, it was not until 2024 that TIL therapy was officially approved as a T cell therapy for refractory melanoma by the US FDA [2].

During those early years of TIL therapy development, one major advancement came from the company Iovance: a T cell culture method that significantly reduced cell culture time and enabled cryopreservation of TILs. With this optimized TIL production process, Iovance was able to reduce the production time of a TIL therapy dose from several months to just 22 days. These cells demonstrated strong efficacy and solid response rates, comparable to earlier NCI data.

Thanks to this standardized process, stable TIL products were successfully expanded from different tumor types—including melanoma, cervical, head, and neck cancers—with a success rate of >90%. These results underscore how overcoming chemistry, manufacturing, and controls (CMC) challenges were key to transitioning TIL therapy from a research concept into a viable product.

STRONG CLINICAL OUTCOMES OF TILs IN REFRACTORY MELANOMA

Based on data from Iovance, their approved TIL therapy for relapsed or refractory melanoma has a strong overall response rate (>36%) and long duration of response (>30 months) [3]. These numbers are particularly meaningful considering patients in this group typically have a short life expectancy.

The impact is even greater when moving TIL therapy from a last-line treatment to a frontline option. When Iovance combined its TIL therapy with PD-1, the overall response rate jumped from about 30% to >85% [4]. This improvement suggests that TIL therapies have the largest impact on patient outcomes when used in earlier

stages of treatment and in combination with PD-1 immunotherapy.

In a separate study on patients with advanced melanoma that failed prior anti-PD-1 treatment, TIL therapy showed improved patient outcomes compared to immune checkpoint inhibitors. The TIL therapy group had an overall response rate of 48.8%, while the rate from the CTLA-4 checkpoint inhibitor group was 21.4%. Progression-free survival was similarly improved, about 7.2 months with TILs treatment compared to 3.1 months with CTLA-4 [5].

EXPANDING TIL-BASED TREATMENTS BEYOND MELANOMA

Beyond using TILs for melanoma treatment, cervical cancer has also shown positive responses according to Iovance data. As a last-line treatment, nearly 44% of cervical cancer patients responded to TIL therapy, with a 7.4-month duration of response [6].

Iovance has additionally demonstrated promising results in non-small cell lung cancer. As a last-line treatment, a solid overall response rate of around 21.4% was observed, along with a >8.2-month duration of response [3]. More strikingly, when used as a first-line therapy, the overall response rate increased to approximately 80% for treatment of naïve patients [7].

TIL therapy has also been tested in colorectal cancer, cholangiocarcinoma, and breast cancer, each showing promising clinical responses.

ADDRESSING CHALLENGES IN CMC DEVELOPMENT OF TIL THERAPY

TIL therapy development starts by collecting tumor samples through surgical excision. Small pieces of a tumor (1–2 g) are then shipped to the manufacturing site, where they are dissected into even smaller pieces. The TILs then go through two stages of amplification: pre-rapid expansion phase and rapid expansion phase. In the first stage, TILs are allowed to migrate out from the tumor tissues. Next, TILs are activated by adding cytokines, and then a bioreactor is used to expand the cells. Once the desired cell number is reached, the cells are cryopreserved and shipped back to the hospital.

When developing CMC for TILs production, a key challenge is collection of the tumor sample. Sometimes, the tumor location is not ideal—some sites are prone to contamination—while others don't have sufficient T cell infiltration. Once the tumor samples are collected, it's essential to maintain sterile conditions during transportation to the manufacturing site and throughout processing.

TIL manufacturing may use animal- or human-derived raw materials, which adds further challenges. A procedure for how these materials will be controlled and evaluated for risks prior to entering the manufacturing process must be established. Improving material risk level assessments, quality evaluations, supplier screenings, and quality agreements is one strategy to minimize potential contamination.

There are also challenges commonly faced with process control. While the cells need to be expanded 1,000–10,000-fold, it's also crucial to maintain high cell viability and low exhaustion marker levels. The choice of bioreactor and defined process parameters will heavily influence the ability to maintain optimal culture conditions. Therefore, identifying critical quality attributes (CQAs), setting reasonable process parameter control, and conducting process testing early in development is key to establishing a robust process.

Given TIL therapy is essentially a mixture of different T cells, there are unique challenges when it comes to quality control. Unlike CAR T or TCR-T cells, which target a single antigen pathway, TILs have multiple targets. This inherent variation can pose

difficulties on defining a potency assay that truly reflects the product. Quality control attributes and potency assays should be developed early in the product lifecycle so they can be validated during Phase 1 and Phase 2 clinical trials.

Finally, product stability is another major consideration, since TILs are living cells. It is crucial to develop a freezing formulation that preserves the cells effectively and to confirm with stability studies that thawed cells still perform as expected after extended frozen periods (~1 year).

OVERVIEW & SAFETY CONSIDERATIONS OF FEEDER CELLS IN TIL CULTURE

In manufacturing, TILs come into contact with feeder cells during cell expansion. The two main types of feeder cells used for TIL development are engineered cell lines and allogeneic PBMCs. Regardless of the feeder cell source, both must undergo inactivation, cryopreservation, and release testing. Control strategies to ensure that the raw materials used in the TIL culture are safe and robust will depend on the type of feeder cell.

For engineered cell lines, the K562 cell is often used to generate a working cell bank for future production. With engineered cell lines, it's essential to ensure the final product does not contain cancer cells that weren't successfully deactivated.

The processes for allogeneic PBMCs are a bit simpler, but critical considerations remain. Since PBMCs come from donors, strategies must be in place to detect viral contamination persisting in the final product that could potentially infect the patient.

CRYOPRESERVATION OPTIMIZATION & RISK CONTROL STRATEGY

Turning to product stability, GRIT has developed proprietary assays to optimize the cryopreservation process so feeder cells retain most of their functionality after thawing. These assays evaluate recovery yield, recovery variability, and proliferation when feeder cells come into contact with the TIL product (Figure 1).

For the risk control strategy, GRIT starts with the quality target product profile (QTPP), which defines key attributes such as the expected dose, stability, strength, and overall quality. Based on these targets, CQAs and potential CQAs (pCQAs) are developed through risk assessment combined with experimental work. Finally, a control strategy that defines the necessary in-process controls and release testing can be established.

DEVELOPING POTENCY & QUALITY STANDARDS FOR TIL THERAPIES

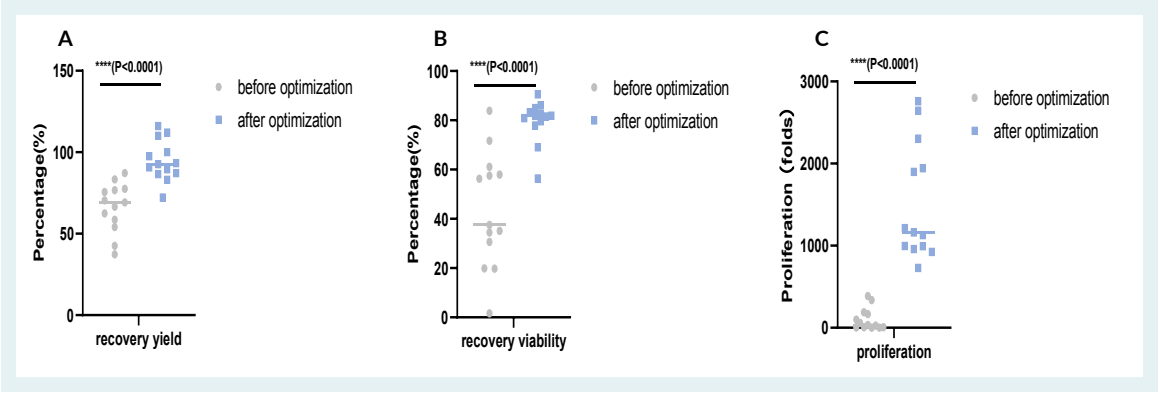
When developing cell therapies, it's crucial to define robust quality standards including appearance, identity, purity, dose, potency, and safety of the product. The development of potency testing is often a hurdle for cell therapies—and especially so for TIL products. Unlike CAR T or TCR-T cell therapies, which have a well-defined antigen target, potency assays for TIL therapies requires a different strategy.

TILs suffer from significant batch-to-batch variability due to the different types of tumor cells and varying population makeup. On top of that, the multiple mechanisms of action that TILs use to fight tumors are difficult to characterize and the target of action is typically unclear because multiple antigen targets are involved. Iovance experienced this challenge firsthand: although their BLA filing was in 2020, their product didn't launch until 2024 due to challenges with the potency assay.

To account for several factors, a matrix approach has been used, where a combined set of assays can provide a comprehensive and reliable measure of potency. These variables underscore why defining

FIGURE 1

Optimization of feeder cell cryopreservation using GRIT's proprietary assays, demonstrating improved recovery yield (A), reduced variability (B), and sustained proliferation (C) upon contact with TIL products.



quality standards as early as possible is crucial—this includes assessing T cell activation, cytokine release, and cell killing. The results should correlate with clinical study data. Quantitative indicators and a well-characterized control group are essential to properly evaluate clinical efficacy.

EXPLORING ADVANCED TIL PRODUCT PLATFORMS & GRIT'S PIPELINE

GRIT has three different platforms used to generate TIL products: the target discovery platform ImmuT Finder®, gene editing tools StaViral® and KOReTIL®, and the manufacturing platform StemTexp®.

ImmuT Finder utilizes high-throughput screening to identify genes that can either positively or negatively regulate the TIL product. StaViral is used for GMP retrovirus vector production at high-quality and low cost, while KOReTIL is a CRISPR-based technology for gene knockout with a rate of >90%. StemTexp is a manufacturing process designed to enrich the population of memory T cells.

GRIT's first product, GT101, is currently in Phase 2, with plans to file a BLA in the coming years. A next-generation product, GT201, is based on a retroviral vector gene editing system that introduces an engineered cytokine into TILs. This product has cleared IND approval in both the USA and China and is currently in Phase 1 studies. The GT300 products were developed to perform a double knockout of pro-exhaustion genes in TILs to target cervical and ovarian cancer. This series is currently in the IND preparation stage. Additionally, GRIT's universal product GT719, an allogeneic cell therapy targeting hematologic malignancies and autoimmune diseases, is currently in the investigational advanced therapy medicinal product stage.

CLINICAL EFFICACY HIGHLIGHTS OF GT101 TIL THERAPY

GT101 therapy is based on GRIT's proprietary manufacturing process. GRIT has completed over 400 TIL batches with a success rate exceeding 90%. As shown in Figure 2, the manufacturing process enriches central memory T cells, which have been clinically shown to correlate strongly with efficacy.

In preclinical studies, promising efficacy was observed in patient-derived organoid (PDO) models, demonstrated by both tumor cell killing and IFN-γ release assays (Figure 3).

GRIT has also completed a Phase 1 study in China, which involved 14 patients—11 of whom had cervical cancer, two with lung cancer, and one with melanoma. Across these patients, a 95% manufacturing success rate was achieved with an average dose level of 3.8×10^{10} cells.

The clinical efficacy of GT101 closely aligned with Iovance's published data, with a 45% overall response rate and a 91% disease control rate (Figure 4A), including one complete response case. The duration of response was also comparable to Iovance's findings (Figure 4B). Additionally, GT101 demonstrated a strong pharmacokinetic (PK) profile (Figure 4C).

ENGINEERED CYTOKINE ENHANCEMENT IN GT201 THERAPY

GRIT's next-generation product, GT201, builds upon GT101 by including an engineered cytokine in TILs to improve persistence in patients. The genetic modification technology is based on the StaViral platform, a retroviral system specifically tailored for TIL products. Using this platform, engineered IL-15 was introduced into TILs, enhancing its survival and tumor-killing activity (Figure 5A).

In contrast to the GT101 product that depends on high-dose IL-2 injections into the patient to support TIL expansion and persistence, the engineered IL-15 with GT201 allows it to function with much less IL-2. Figure 5B illustrates that even after IL-2 withdrawal, a strong T cell persistence in the body was observed.

When designing GT201, losing T cell clonality was a concern since engineered products can lead to the outgrowth of

FIGURE 2

The comparison of central memory T cells (CD8, %) in non-responder (NR), complete responder (CR), and GT101 groups.

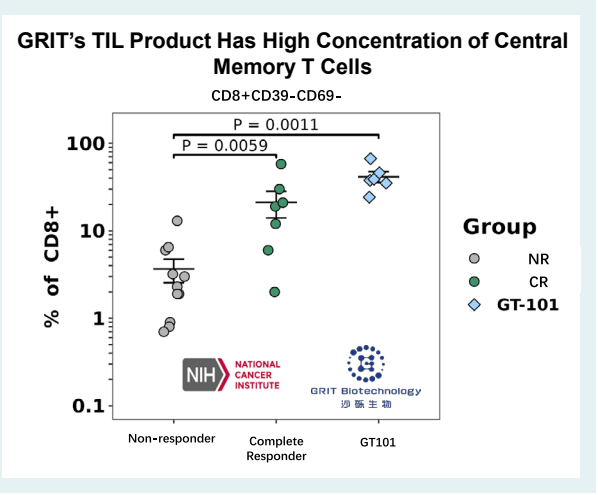
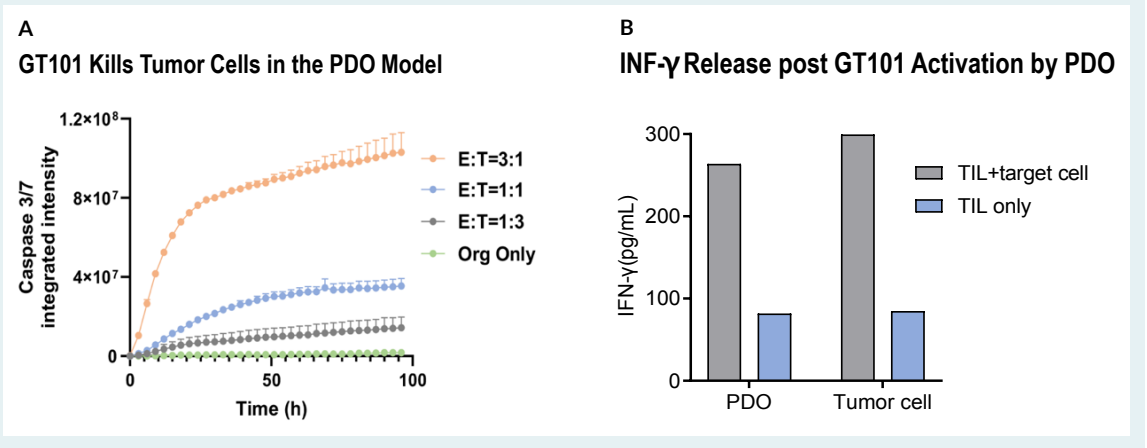


FIGURE 3

Tumor cell killing efficiency of GT101 in the (A) PDO model and (B) IFN-γ release assay.



specific T cell populations. However, a very similar clonality between pre-expansion cells and the final product was observed, indicating preservation of the initial TIL profile. Retention of population diversity is crucial to effectively targeting the heterogeneity of solid tumors.

Additionally, in a cervical patient-derived xenograft (PDX) model used for internal in vivo validation, a strong tumor

FIGURE 4

Clinical performance of the GT101 product, showing (A) overall response rate and disease control rate, (B) duration of response, and (C) pharmacokinetic profile.

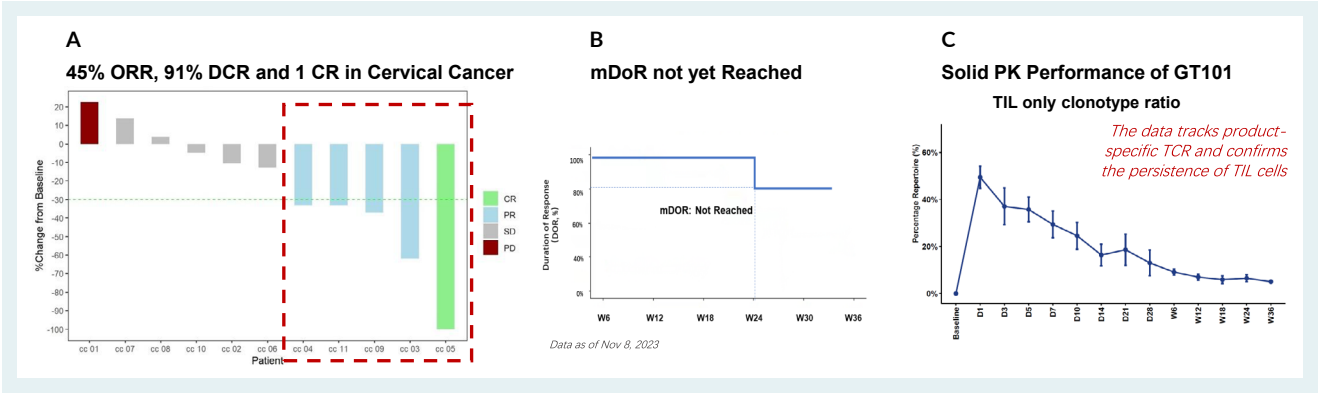
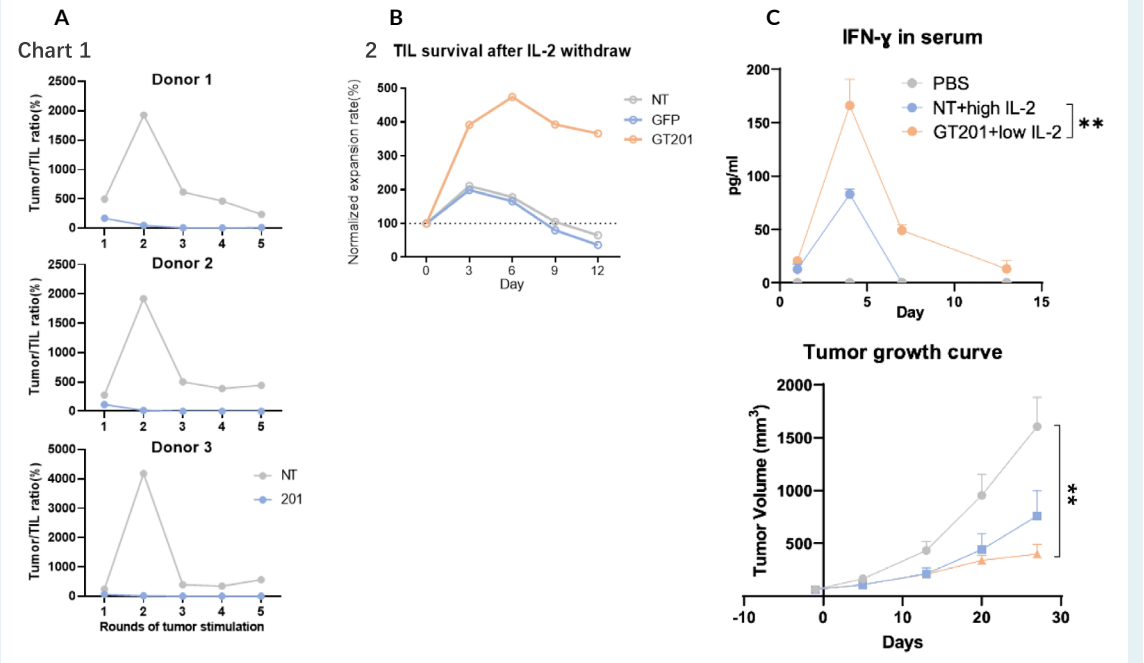


FIGURE 5

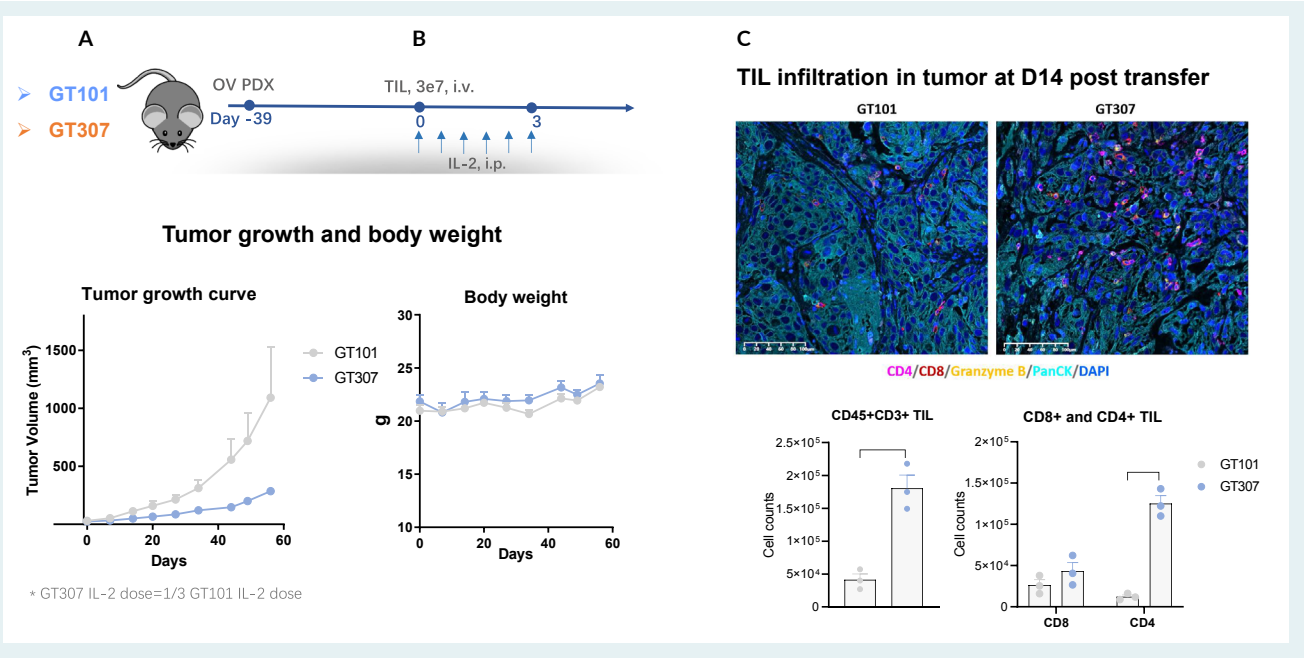
Validation of the functional advantages of GT201.



(A) *In vitro* TIL survival and tumor-killing activity enhanced by IL-15; (B) *in vitro* TIL survival after IL-2 withdrawal; (C) *in vivo* validation results in cervical cancer PDX model: IFN-γ in serum and tumor growth curve.

FIGURE 6

Comparison of (A) tumor growth, (B) body weight, and (C) TIL infiltration into tumors between GT101 and GT307 products based on ovarian PDX model.



control rate and high IFN-γ secretion in serum were observed (Figure 5C).

DOUBLE KNOCKOUT STRATEGY & TARGET DISCOVERY IN GT300 THERAPIES

GRIT's GT300 product series is based on a double knockout approach to enhance TIL functionality. ImmuT Finder is a high-throughput screening platform designed to identify key gene targets that optimize TIL functionality, while KOReTIL employs multiple optimization techniques and achieves knockout efficiency of >90%.

Unlike traditional methods that rely mostly on *in vitro* or purely algorithm-based assays, ImmuT Finder combines both *in vitro* and *in vivo* screening. This integrated approach significantly improves the success rate of finding potential targets. Using this platform, over 100 potential targets were identified. From these, 6 top candidates emerged based on functional assays. The GT300 series is being developed around two of those targets and is currently progressing through IIT-stage studies.

Looking at preclinical data (Figure 6A), the tumor control rate with the GT307 product is substantially improved relative to GT101, while the safety profile, as measured by body weight, remains comparable (Figure 6B). Furthermore, GT307 demonstrates significantly improved TIL infiltration into tumors compared to GT101 (Figure 6C).

In essence, GT307 represents a breakthrough next-generation product with strong commercial potential. While GT101 focuses mainly on cervical cancer and melanoma, GT307's indications are expanding to include ovarian, colorectal, and non-small cell lung cancers.

TRANSLATION INSIGHT

TIL therapy for solid tumors can only be advanced by addressing major CMC challenges such as tumor sourcing, cell expansion, and potency testing. Using proprietary

platforms, GRIT has developed products such as GT101 and GT201 that show strong clinical outcomes and scalability. The StemTexp platform enables TIL enrichment with a high-memory phenotype while maintaining their essential polyclonality (GT101). Building on this foundation, GRIT introduced engineered cytokines into TIL cells using StaViral technology to enhance T cell persistence in the patient's body (GT201). Most recently, the GT307 product is produced with gene knockout tools, including theImmunT Finder that removes undesirable genes, enabling the TILs to better survive and function within the harsh TME.

Q&A

Alex Lei (left), **Sabrina Carmichael** (right)

Q Have you noticed outcome differences between automated and manual expansion systems?

AL We have observed significant differences between automated and manual systems in TIL therapy manufacturing. Automated systems allow us to expand the cells to the desired quantity while maintaining their stem cell-like properties due to continuous monitoring throughout the process.

In contrast, manual systems often face challenges because the final product volume is large, and without automation, it becomes nearly impossible to closely monitor cell types and their condition during expansion. By the time of harvest, the product quality can be quite uncertain.

For these reasons, we strongly believe that automated manufacturing is the way forward for consistent and high-quality TIL therapy production.

Q What are your expectations for future process improvements in the TIL manufacturing workflow?

AL Although most of our system is already automated, there are still some steps that require manual intervention—for example, tumor collection, dissection, and certain parts of the manufacturing process involve a lot of hands-on work.

Ideally, we want to transition from these manual steps to a fully automated process. This would dramatically reduce costs and potentially improve product quality. It is still in early stages, but there are companies actively working on making this a reality—just like how Iovance transformed Rosenberg's original concept into a commercial product.

Q Which technologies have you seen successfully integrated into TIL workflows, either upstream or downstream?

SC On the upstream side, IL-2 and IL-15 are two critical cytokines for TIL expansion, and Cytiva™ offers these cytokines in a variety of formats and sizes, including lyophilized powders and liquid syringes, designed to fit a wide range of workflows.

Regarding equipment, the Xuri™ cell expansion system is particularly well-suited for the rapid expansion phase (REP) of TIL manufacturing thanks to its scalability, automation capabilities, and support for culture intensification. Using this bioreactor, we've reported on a GMP-aligned high-yield, high-viability TIL expansion protocol, which includes sterile sampling capabilities.

Also, the VIA Thaw™ device from Cytiva™ is used for thawing frozen cryobags. Many laboratories, including ours, often freeze the product between the pre-rapid expansion and rapid expansion phases. The VIA Thaw™ automated thawer is a waterless device, making it perfect for cleanroom environments and GMP manufacturing.

For downstream processing, we have the Sefia™ S-2000 system, which works alongside the FlexCell protocol software. This is an automated, closed processing system paired with a dedicated protocol, designed to work seamlessly together for harvesting and final formulation of the product. One of the key benefits of this system is its capacity to handle up to 10 L per run, which offers significant scale-up potential for TIL workflows.

Cytiva™ also offers the VIA Freeze™ controlled-rate freezer, which is liquid nitrogen-free. It can freeze samples in various formats, including cryobags and cryovials. The VIA Freeze™ freezer delivers consistent freezing performance and is GMP-compatible, addressing the regulatory needs of cell therapy manufacturers.

Q Where do you see the most variability in the process, and how do you manage it?

AL The greatest source of variability in our process is the initial material—the patient's tumor. To manage this, we conduct extensive process development to identify optimal tumor samples for use. This also requires close collaboration with physicians to ensure we can access the right tumors.

Additionally, the composition of the transport media used to preserve the tumor during shipment is critical. Developing and optimizing this media early in the process is essential because, without high-quality starting material, no matter how advanced your manufacturing process is, the final product quality may be compromised.

Q What are some of the biggest challenges in scaling TIL manufacturing?

AL Whether scaling up or scaling out TIL manufacturing, there are always challenges in maintaining optimal cell conditions throughout the expansion

and harvest processes. Scaling up means increasing the cell dose from levels comparable to CAR-T or TCR-T cell therapies—usually in the millions or tens of millions—to the much higher doses required for TIL therapy, which can be in the billions or tens of billions of cells.

This is where bioreactors such as the Xuri cell expansion system become crucial. They allow us to tightly control culture conditions—through feedback mechanisms or continuous nutrient and media supply—to enhance the final product profile.

Scaling out, on the other hand, means increasing the number of parallel manufacturing processes to produce more batches simultaneously. This is important to maximize production capacity, reduce manufacturing costs, and ultimately lower the price for patients. Closed, automated systems are instrumental in enabling efficient scaling out, as they allow for multiple, simultaneous production runs.

Q Does Cytiva offer monitoring or analytics tools to control TIL expansion more precisely?

SC We have our Chronicle™ automation software, which is a cloud-based platform that can monitor your workflow systems throughout the production runs. The software has different components, such as allowing you to monitor the current run, execute electronic standard operating procedures, and create batch records that include complete data sets. You can also take inventory of your materials, which is very important for GMP compliance.

Notably, Chronicle software can be used to connect not just with Cytiva systems, but with third-party systems as well. This is especially beneficial when working with FlexFactory™ platforms provided by our enterprise solutions team. These FlexFactory systems include third-party equipment, such as analytical instruments, as well as traditional laboratory equipment, such as environmental monitors and incubators.

This integration means you can automatically load your cell counts or phenotype data from analytical instruments directly into your batch records using Chronicle software.

Q What are the key considerations when selecting a bioreactor for TIL production?

AL When selecting a bioreactor, we focus on whether it can support both scale-up and scale-out. For example, with the Xuri system, we can adjust various parameters to meet our final product needs.

Since the expansion phase for TIL manufacturing is much longer compared to CAR T cells, controlling the bioreactor—such as managing media input and output—is critical. Another important consideration is to figure out how many bioreactors can be used simultaneously to produce the therapy when scaling out.

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BIOGRAPHIES

Alex Lei is a seasoned expert in the field of solid tumor immunotherapies. He holds a PhD from the University of Southern California, CA, USA and currently serves as the Chief Technology Officer (CTO) at GRIT Biotechnology. Prior to this role, Dr Lei held leadership positions including CTO at TCRCure Biopharma Corp., as well as Senior Manager roles at Johnson & Johnson and Henlius Biotechnology. Over the course of his career, Dr Lei has led teams through multiple IND submissions and clinical trials targeting solid tumor interventions, demonstrating deep expertise in translational oncology and regulatory strategy.

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Sabrina Carmichael is the Global Technical Leader for the Cell Therapy Fast Trak team at Cytiva. She holds a master's degree in Biotechnology from John's Hopkins University, MD, USA. Since joining Cytiva in 2017 as an R&D Scientist, she has played a role in developing innovative scientific solutions that drive the company's impact in the life sciences sector. In 2019, Sabrina transitioned to the Fast Trak team, where she currently leads the global training program, providing hands-on, immersive training for clients and partners. This program offers comprehensive insights into Cytiva's advanced cell therapy manufacturing platform, covering the entire end-to-end cell therapy workflow. Through her strategic guidance, the

program equips customers with essential skills to harness Cytiva's technology effectively in clinical and commercial settings. Beyond training, Sabrina directs complex process development projects, collaborating with customers to design and refine novel approaches in cell therapy manufacturing.

Sabrina Carmichael, Global Technical Leader, Cell Therapy Fast Trak, Cytiva, USA

AUTHORSHIP & CONFLICT OF INTEREST

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You've read the science– now see the solution

TIL therapy manufacturing

Robust, automated systems with the flexibility you need to tackle tomorrow's challenges.

Tissue process → Collection → Cryopreservation

VIA Thaw™ instrument

Thawing

Thaw cryopreserved tissue samples with the automated and GMP-compatible VIA Thaw™ dry thawer.

Pre-rapid expansion protocol (REP)

Harvest and formulation

These two steps can be performed with either platform:

Sepax™ C-Pro system

- Sepax™ C-Pro offers a gentle, closed-system harvest that supports high cell viability and purity while enabling scalability. Its modular design and protocol-based automation make it ideal for decentralized or early-phase workflows.

Sefia Select™ system

- Sefia Select™ enables automated cell concentration, buffer exchange, and precise formulation in a closed, GMP-compliant workflow. With a 10 L capacity per run and integrated workflows, it supports mid-scale manufacturing and helps accelerate time-to-patient.

Isolation

Sepax™ C-Pro system

The isolation step can be performed with either platform:

- Sepax™ C-Pro system supports a semi-automated workflow ideal for cell washing and feeder cell preparation, providing consistent and gentle handling of cell suspensions.
- Sefia Select™ system enables an automated approach for cell washing and magnetic selection, providing flexibility for targeted enrichment within TIL workflows.

Sefia Select™ system

REP

Xuri™ W25 system and reagents

Xuri™ W25 system enables high-viability TIL expansion on a scalable, automated platform that supports culture intensification and sterile sampling.
For advanced strategies like CAR-TILs, Xuri™ bioreactor integrates seamlessly into lipid nanoparticle (LNP) workflows empowering you to scale innovation without compromise.

Cryopreservation

VIA Freeze™ range

Cryopreserve cellular product with the GMP-compatible VIA Freeze™ controlled-rate freezer, available in three sizes that can support both cryobags and cryovials.

Automation and connectivity

Chronicle™ automation software supports process monitoring, traceability, and integrated manufacturing workflows.

Infusion ← Reformulation ← Thawing

Designed to increase productivity

Sefia Select™ system and MagnetSelect application software:

- Fully automates magnetic isolation from apheresis to resuspension.
- Requires one-third less operator touchpoints and fewer labor hours compared to semi-automated workflows (Sepax™ C-Pro system and CliniMACS Plus).

Request your personalized demo today

Manual process	Semi-automated	MagnetSelect
Apheresis washing: remove platelets and reduce volume	Apheresis washing + bead incubation + bead washing	Apheresis washing + bead incubation + bead washing + magnetic cell isolation + post washing
Bead incubation: binding of beads to cells		
Bead washing: remove unbound beads		
Magnetic cell isolation of targeted cells	Magnetic cell isolation	
Post washing: exchange media and concentrate cells	Post washing: exchange media and concentrate cells	

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Temperature matters: fluid management for cell-based applications

Jonathan Haider, Product Line Manager, Fluid Management, Single Use Support GmbH

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Cell-based manufacturing faces challenges that can compromise product quality and efficiency, including sedimentation, inconsistent homogenization, and inaccurate aliquotation. Effective temperature regulation is essential to preserving cell viability throughout processing. This poster presents data evaluating a closed, temperature-controlled fluid management approach to mitigate these issues.

TEMPERATURE-CONTROLLED HOMOGENIZATION FOR CONSISTENT CELL DISTRIBUTION

During filling, viable cells tend to sediment and clump in the source bag, leading to inconsistent sampling, inaccurate aliquotation, and compromised product quality. This challenge is compounded by the use of dimethyl sulfoxide (DMSO) as a cryoprotectant, since prolonged exposure can significantly reduce viable cell density, as shown in Figure 1. Addressing these issues requires a homogenization solution that can reliably maintain uniform cell distribution while minimizing thermal stress.

To evaluate a potential solution, Single Use Support conducted two studies using RoSS.PADL, a gentle kneading device for source bag homogenization.

In the first study, a 2 L bag (1.25 L fill volume) containing *Saccharomyces cerevisiae* at 10^8 cells/mL was used. Samples were drawn in triplicate at five intervals, demonstrating no significant variation in viable cell counts across all time points. A second study using a larger 20 L bag (8 L fill volume) produced comparable results, confirming the reproducibility of cell homogeneity at scale. The depth and frequency of RoSS.PADL's kneading mechanism can be adjusted to optimize homogenization for different bag sizes and cell suspensions. The impact of RoSS.PADL homogenization on uniform cell distribution is illustrated in Figure 2.

To reduce DMSO-induced cell damage during processing, RoSS.PADL integrates a temperature-control element beneath the source bag (Figure 3). This thermal plate can be cooled to 2 °C, enabling a stable source bag temperature of 2–8 °C throughout mixing.

AUTOMATED COOLED FILLING TO SUPPORT PRECISION AND SCALABILITY

Following homogenization, cell suspensions must be filled into final containers rapidly and consistently while minimizing temperature fluctuations. Manual processes can result in inconsistent fill volumes, delayed freezing, and increased DMSO exposure time (Figure 1), affecting product quality and cell recovery.

To address this, Single Use Support has developed RoSS.FILL, an automated, closed-system filling unit equipped with cooling and insulation throughout the fluid path. Tubing is enclosed in form-fitting cooled channels and insulated materials, keeping the entire filling pathway, including the venting bag, down to 2 °C. Additional temperature sensors continuously monitor the fluid during transfer. Filled bags rest on phase change materials maintained at 2 °C, ensuring the product remains within the optimal temperature range until freezing (Figure 4).

SUMMARY

The studies presented here demonstrate that temperature-controlled homogenization with RoSS.PADL and cooled, automated filling via RoSS.FILL support a standardized, closed process for cell aliquotation. Together, these technologies reduce DMSO exposure, improve cell viability, and enhance batch consistency. By enabling reproducible performance across volumes and minimizing operator intervention, this integrated fluid management approach lays the foundation for scalable, GMP-compliant cell processing.

Figure 1. Effect of dimethyl sulfoxide (DMSO) exposure time on viable cell density.

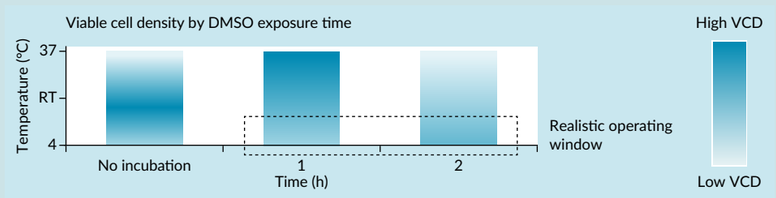

Figure 2. Homogenization of cells in the source bag before (left) and after (right) mixing with RoSS.PADL.

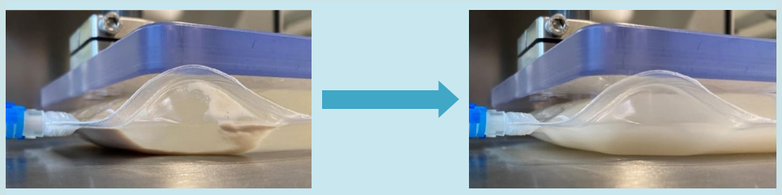

Figure 3. RoSS.PADL system with kneading mechanism and integrated thermal plate.

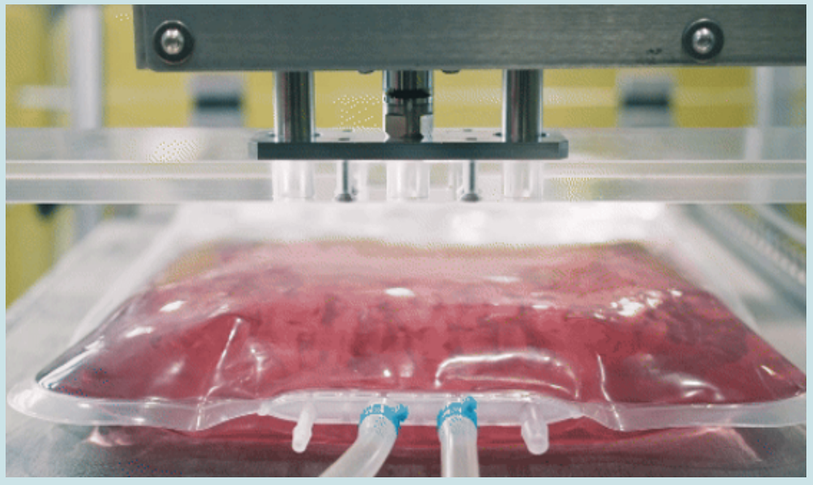

Figure 4. RoSS.FILL filling unit.

Jonathan Haider is the Product Line Manager for Fluid Management at Single Use Support GmbH. He has a background in mechanical engineering and business, and he brings his expertise to the field of fluid management solutions.

CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

Avoiding slowdowns on the cell and gene therapy development pathway

William E Janssen and Scott R Burger

VIEWPOINT

"By fostering structured educational exchanges, embedding process engineering expertise early, and investing in modular, automated technologies, the field can reduce inefficiencies..."

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INTRODUCTION

Process development of cell and gene therapy (CGT) products, from their origins in academic research labs to licensure and commercialization, involves multiple technology transfer steps. This is a critical but often problematic transition step in the lifecycle of CGT products. The science underpinning many novel CGT products originates in academic research laboratories, but reaching commercialization requires transfer to industry, for process development and controlled manufacturing. Subsequent technology transfers—from process development to GMP manufacturing, from one

GMP manufacturing site to another—take place between industry laboratories and facilities, i.e., industry-to-industry. The first transfer, however, is a transition between two distinctly different cultures, academic research-and-industry manufacturing, and so presents unique challenges. Here we examine the primary obstacles encountered in early-stage technology transfer and process development and propose some solutions to help bridge the gap between academic innovation and industrial product realization.

CHALLENGES IN TECHNOLOGY TRANSFER & PROCESS DEVELOPMENT

Process scalability and standardization

Academic research commonly relies on manual, open-system, small-scale techniques with limited process control. These methods allow researchers the necessary flexibility, but are unsuitable for clinical scale GMP manufacturing, necessitating process development to establish a suitably scaled process capable of validation. The lack of controlled, consistent procedures at the transferring research laboratory hampers adoption by the industry facility and creates obstacles for process development.

The diversity of CGT products adds further complexity, as some novel product types may require unique manufacturing paradigms. Many emerging processes are not supported by currently available closed and automated equipment, forcing reliance on predominantly manual methods that are often only partially closed.

Materials quality and supply chains

Academic laboratories commonly use materials suitable for research but not for use in GMP manufacturing. Research-use-only cytokines, enzymes, or media may not be available in an equivalent GMP version, or the supply may have long lead times, delaying and complicating technology transfer and process development. Sourcing cellular starting material for technology transfer and process development studies presents difficulties as well.

Gaps in expertise and expectations

Academic research and industry GMP are different cultures, with different backgrounds. Academic researchers are experts in science but usually have limited familiarity with product development, and the operational and regulatory requirements of GMP manufacturing. On the other hand, industry-based technology transfer teams from CDMOs often lack in-depth knowledge of the relevant preclinical methodology and data, necessitating training in techniques and introducing elements of trial and error into the process. This expertise gap gives each party in the transfer very different expectations of the other, resulting in miscommunications and inefficiencies during transfer.

Documentation and data

A clear, detailed description of the processes being transferred, supported by examples of expected data, is central to technology transfer and process development, to enable drafting GMP documentation. Academic research laboratories sometimes have difficulty providing the necessary details. Research laboratory notebooks, properly kept, are sufficient to support publications, but often lack the level of detail needed to create batch records or regulatory submissions. Incomplete laboratory notes or data are an even greater problem.

This reflects a fundamental difference between research and GMP manufacturing. Research involves a flexible approach, changing conditions to test one hypothesis, then another. GMP-compliant operations, on the other hand, require meticulous

recordkeeping, and following standard operating procedures to ensure that processes are done exactly the same way, again and again. This cultural divide is often apparent in the differences between academic research and industry GMP documentation practices, creating friction in technology transfer and process development.

Regulatory misalignment

Academic investigators may have only limited exposure to the regulations governing CGT products. Early regulatory engagement is uncommon in academia, but without it, investigators may not be aware of the specific information they are expected to establish, leading to delays during technology transfer and beyond.

POTENTIAL SOLUTIONS

If CGTs are to become accessible to the many patients who could benefit from them, we must address the obstacles that delay or derail technology transfer and process development. We propose the following potential solutions:

Education, for all parties involved

- Curriculum development: universities should integrate product development, specifically, GMP fundamentals, regulatory science, biomanufacturing principles, and process development, into biomedical graduate programs. Early exposure would help trainees appreciate the requirements and expectations of translational research. In addition, seminars on these topics should be given periodically, to reach faculty-level researchers;

- Exchange programs: fellowships allowing academic scientists to rotate through GMP facilities, and vice versa, would foster mutual understanding;

- Regulatory consultation incentives: policies that provide academic labs with early, subsidized access to regulatory experts—funded by industry or nonprofit consortia—would encourage alignment with industry expectations from the start.

Academic-industry partnerships

Greater interaction is needed between the researchers who drive early-stage development and those responsible for designing and implementing GMP manufacturing processes.

- Shared personnel: introduction of personnel who possess a process engineering skillset into the CGT development process, as early in that process as possible, would facilitate effective planning for early phase CMC, and for further evolution of the CMC as development progresses. This could be done by embedding industry-trained process engineers into academic labs, and academic scientists into industrial process development teams. This would reduce cultural and technical divides as well;

- Pre-competitive consortia: industry and academia could collaborate in neutral forums to have focused discussions on maturing laboratory methods into closed, automated, and GMP-compliant manufacturing programs. Much smaller and more narrowly focused than CGT conferences, these forums could take the form of online workshops or in-person meetings modeled after Cold Spring Harbor or Gordon Conferences.

Modular technology

Current large-scale production systems offer some value, but they are not universal solutions and face scalability limitations. A

more sustainable vision is a modular and flexible system supported by a common software platform. Such software would allow seamless communication among equipment from different manufacturers, integrating components such as closed bag and tubing systems, cell selection devices, washers, bioreactors, and finish and fill devices.

Development of modular plug-and-play systems for common CGT manufacturing unit operations would enable smoother scaling from bench to clinic. Miltenyi Biotec's MACS cell selection technology is a good example of the effectiveness and value of such tools. Immunomagnetic cell selection is performed at mouse scale in research laboratories using the MiniMACS and translates to clinical scale relatively easily on the CliniMACS.

A platform with interchangeable components could create a fully automated, closed manufacturing system capable of evolving with the product's development. This adaptability would not only ensure product consistency and scalability but also support the transition from early clinical trials to commercial production without requiring complete redevelopment of manufacturing infrastructure.

CONCLUSION

The path from proof of concept to commercialization in CGT is complex, with significant obstacles to scaling up and scaling out manufacturing. However, these barriers are not insurmountable. By fostering structured educational exchanges, embedding process engineering expertise early, and investing in modular, automated technologies, the field can reduce inefficiencies and accelerate access to transformative therapies.

BIOGRAPHIES

William E Janssen has worked for more than 30 years at translating lab bench models into cell and gene therapy products for administration to patients. In the course of these efforts, he has developed and refined methodologies for all aspects of cell-based therapy from collection, through manufacturing and administration, including regulatory aspects. Dr Janssen has also been responsible for facility design, drug master file creation, development of staff training programs, process engineering, technology transfer, SOP development, process validation, comparison studies and integration of processes, equipment, and raw materials. He has also been both a proponent and implementer of informatics solutions for management of cell and gene therapy development and manufacturing data.

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Scott R Burger is the Founder and Principal of Advanced Cell and Gene Therapy, a consulting firm specializing in cell and gene therapy product development, providing expertise on manufacturing, regulatory, and strategic aspects of these products. Dr Burger has over 30 years of experience developing cell and gene therapy products and has consulted for over 160 companies in North America, Europe, Australia, and Asia, advising on a wide range of cell therapy and gene therapy products for immunotherapy and regenerative medicine applications. Dr Burger has consulted on over 100 regulatory submissions for cell therapy or gene therapy products at all stages of development. He has served as an expert witness in cases involving cell and gene therapy intellectual property, commercialization, FDA regulatory affairs, and GMP compliance, and as a subject matter expert for NIH-NHLBI, CIRM, PACT, and DMRDP review panels. He has been an invited speaker at internal FDA

workshops and is frequently asked to speak on cell and gene therapy manufacturing and regulatory topics at international conferences. A graduate of the University of Pennsylvania School of Medicine, Philadelphia, PA, USA, Dr Burger completed training in clinical pathology and transfusion medicine at Washington University in St Louis, St Louis, MO, USA and is author of over 200 scientific publications and presentations, and recipient of numerous honors and awards.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

Bridging preclinical to commercial manufacturing in cell therapy: mind the gap

Ashwin Srinivasan Kumar, Sabry Hamza, and Jaichandran Sivalingam

VIEWPOINT

"A phase-appropriate approach for cell therapy manufacturing is essential..."

Cell & Gene Therapy Insights 2025; 11(8), 1037–1044 · DOI: 10.18609/cgti.2025.119

Translating innovative cell and gene therapy products from bench to bedside remains hindered by significant translational barriers. Developers face a dilemma: prioritize speed-to-clinic via simple, open-manufacturing systems for a first-in-human trial, or speed-to-market through investments in scalable, compliant manufacturing platforms. Reliance on open systems during early manufacturing, while permissible under Phase 1 cGMP guidelines, poses critical sterility and scalability risks that impede the path to commercialization [1].

The strategic question becomes: how can we close the gaps between preclinical, process development (PD), and commercial manufacturing to ensure seamless

transition while maintaining phase-appropriate practicality and regulatory compliance?

UNDERSTANDING THE CMC, REGULATORY, & MANUFACTURING REQUIREMENTS AT DIFFERENT STAGES OF PRODUCT LIFE CYCLE

Preclinical focus centers on demonstrating product safety, toxicity, pharmacokinetics, efficacy, and mechanism of action (MoA) in GLP-accredited facilities [2]. Manufacturing is typically small-scale, using open platforms and research-grade reagents. While cheaper, such reagents (e.g., less purified research-grade viral vectors or FBS) complicate comparability studies and hinder transition to cGMP-compliant processes that require GMP-grade viral vectors or clinical-grade, serum-free, or xeno-free medium. Regulators advise using such defined media early as a key risk mitigation strategy to minimize risk of adventitious agents and batch-to-batch variability.

PD supports CMC documentation in Module 3 of the Common Technical Document for INDs in the USA or the EU Investigational Medicinal Product Dossier (IMPD). While the US FDA guidance allows certain exemptions from 21 CFR Part 211 for Phase 1 clinical trials, fundamental GMP principles from 21 CFR Part 210 still apply: use of GMP-grade reagents, standardized procedures, well-defined processes, calibrated and qualified equipment and facilities, trained personnel, phase-appropriate vendor and material qualification, and demonstrated preliminary data on product safety, identity, purity, and potency [3,4]. As Phase 1 trials primarily demonstrate safety, with efficacy as a secondary endpoint, having flexibility with non-commercial ready, yet robust manufacturing workflows would be most appropriate for the majority of early-stage biotechs. Progression to later trials, however, requires a transition towards closed, scalable workflows and deeper process knowledge to support commercial manufacturing.

Analytics also evolve across stages: early-stage potency assays may qualitatively demonstrate biological activity, but often lack the robustness, specificity, and reproducibility required for lot release, mandated by specifications in ICHQ6B [5]. Bridging studies are often needed to compare legacy and improved analytical assays as part of continuous improvement. It is inevitable that early decisions made in the PD stage may have implications for the clinical pipeline development, underscoring the need for a clear roadmap for process upgrade before pivotal trials and commercial manufacturing.

Full cGMP compliance is mandatory by commercial stages. Adopting validated, closed-system workflows with defined proven acceptance range (PAR) and normal operating range (NOR) is a key strategy to mitigate risk during the manufacturing process and enhancing commercial viability. Processes, equipment, and facilities must be validated for scale-up and scale-out to accommodate increasing manufacturing demands. Analytical methods must be developed in line with ICH Q14 guidelines and validated as per ICHQ2 guidance [6,7]. Materials and vendors must be qualified according to 21 CFR Parts 210 and 211 [3,4], US and EU Pharmacopeia ancillary materials standards [8,9], and relevant biologics-specific requirements outlined in 21 CFR Parts 600-680 [10]. Robust procurement, supply chain and cold-chain logistics, chain-of-custody, and data management systems are essential for regulatory approval and inspections (Table 1).

STREAMLINING THE PATH FROM ACADEMIA TO INDUSTRY

Embedding commercial translation requirements into early PD minimizes costly delays, increasing commercial

TABLE 1

Regulatory and CMC requirements by development phase.

Preclinical phase	Process development/IND	Commercial phase
Research grade reagents	GMP principles (21CFR210)	Full cGMP (21CFR210-211)
Open systems	Phase-appropriate controls	Manufacturing process validation
GLP requirements (21CFR58)	Closed workflows	PAR/NOR set
Small-scale manufacturing	Valid equipment	ICH Q2/Q14 qualified analytical methods
Safety/efficacy	Trained staff	Qualified suppliers
Manual operation	Identity/purity/potency	Supply chain validated

viability. This 'begin with the end in mind' approach requires early alignment on commercial-scale manufacturing strategies such as adoption of automated scale-up or scale-out manufacturing platforms early in development. Integration of digitalization during development eases future manufacturing and streamline data collection. Future-proofing analytical capabilities by incorporating Process Analytical Technologies (PAT) for real-time monitoring during manufacturing enhances product quality control (Figure 1).

PROCESS DEVELOPMENT WITH END IN MIND: QBD

Implementing QbD principles early in PD using multivariate and DoE methodology allows to define the process design spaces and link critical process parameters (CPPs) with critical quality attributes (CQA). Early mapping and control of CPPs via close collaboration between preclinical and PD teams streamlines the transition towards process validation and Process Performance Qualification (PPQ) in later manufacturing phases.

ANALYTICAL DEVELOPMENT IN EARLY PHASES

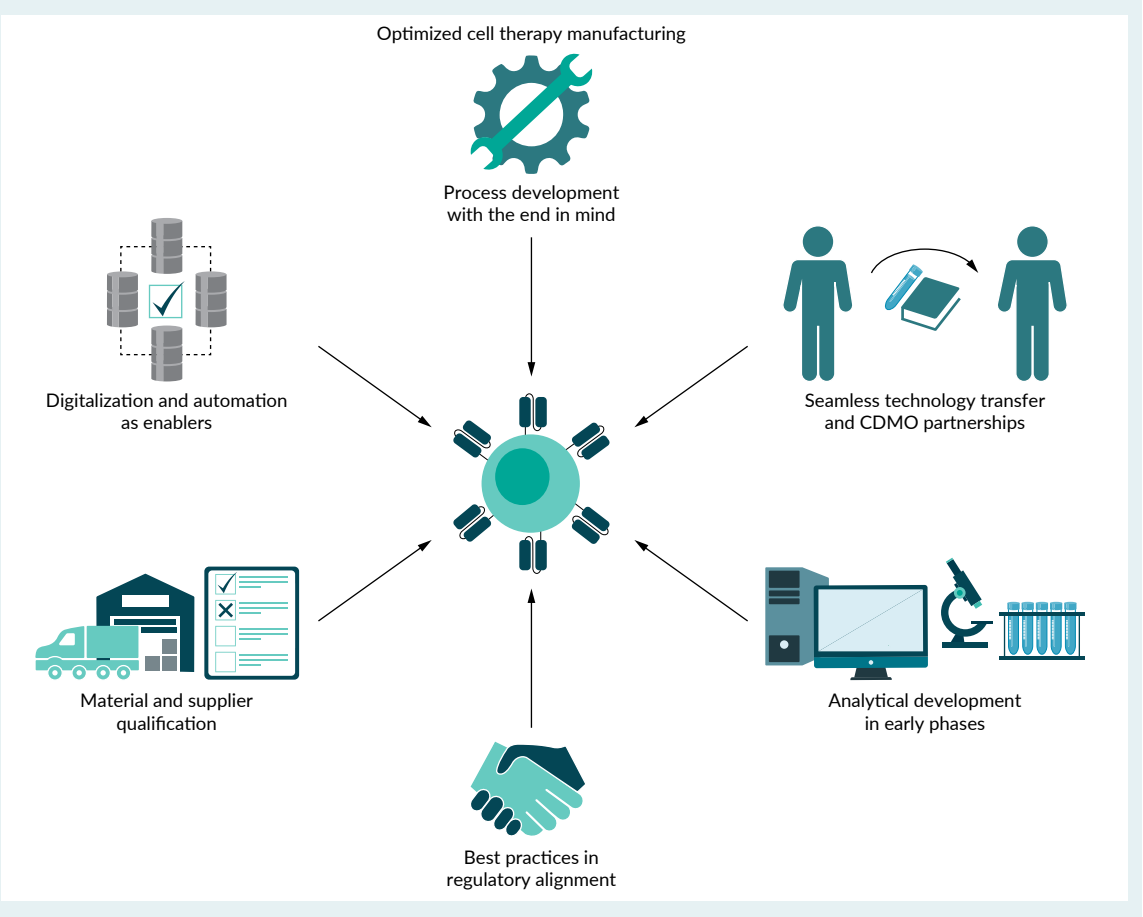
Analytical methods must co-evolve with process understanding. Early adoption of PAT enables real-time monitoring and adaptive control, while establishing a matrix approach to assay development comprehensively captures product potency and MoA for regulatory submissions [11]. These approaches facilitate the integration of machine learning and AI tools aligned with Manufacturing 4.0 principles to enhance product quality assessment and release.

CLOSED SYSTEM MANUFACTURING, SCALABILITY, & AUTOMATION

Early integration of phase-appropriate processes minimizes costly downstream comparability and bridging studies. Harmonizing early-stage development with commercialization demands adopting processes with the end in mind. Utilization of closed-system automated manufacturing platforms that support autologous and allogeneic workflows, offer integrated cell processing and expansion, and can be validated for compliance with 21 CFR Part 11 requirements, facilitating a smoother transition towards commercial scale-out manufacturing. PD work on these platforms establishes a clearer path towards commercial manufacturing without necessitating late-stage comparability studies that regulatory authorities may require if platform changes occur later. Interconnectivity across different manufacturing equipment and digital control systems is equally

FIGURE 1

Early adoption of best practices for commercially aligned cell therapy manufacturing.



important for modular manufacturing workflows and can be achieved with software solutions that provide end-to-end data integration and orchestration.

ADOPTION OF FDA ADVANCED MANUFACTURING TECHNOLOGY

Early adoption of automated manufacturing platforms with FDA Advanced Manufacturing Technology (AMT) designation [12] can accelerate the transition towards regulatory approvals. These can include AI-driven platforms for automated, scalable manufacturing of iPSCs, and large-scale, self-contained 'GMP-in-a-box' systems designed for industrial production of immune cell therapies. Innovative, compact benchtop solutions utilizing microfluidics or other modular approaches provide cGMP-compliant, end-to-end solutions that enable a seamless transition of products from pre-clinical research through to commercial-scale manufacturing.

LEVERAGING EXTERNAL EXPERTISE FOR MANUFACTURING

Early partnerships with CDMOs leverage their PD and commercial manufacturing expertise to accelerate commercialization. Many CDMOs now offer access to established, therapy-class-specific manufacturing platforms that leverage expertise developed across multiple client pipelines, significantly reducing development timelines. This advantage

stems from pre-existing master batch records, validated standard operating procedures (SOPs), qualified analytical methods, completed aseptic validation, experienced personnel, and reliable supply chains. Partnering with such CDMOs offering pre-optimized platforms minimizes upfront PD investments, expedites commercial development, reduces manufacturing failure risk, and lowers costs for IND-enabling studies and clinical production.

SUPPLY CHAIN MANAGEMENT

Validated supply chains are needed to ensure that high-quality raw materials and starting materials are available for manufacturing. Having established and reliable supplier relationships ensures stable and continuous supply of ancillary raw materials for disruption free manufacturing. Identification of alternate materials and their impact on safety, quality, purity, identity, potency and stability of the manufactured product mitigates supply chain risks, ensuring continuity of production and avoiding requirements for comparability studies that may arise due to inadvertent late stage change in raw material utilization. Procedures to identify, establish, and periodically review primary and alternate suppliers should be implemented during early-stage clinical development and are useful for transition towards commercial manufacturing. Validated collection and shipping of healthy or patient starting source materials for manufacturing, chain-of-custody management, and subsequent shipping of manufactured product for clinical administration would be critical for clinical operations.

DIGITALIZATION

Another important area for early adoption is the shift from laborious and tedious paper-based records towards digital solutions for establishing quality management systems (QMS), manufacturing batch records, eSOP, change and deviation management, inventory, workflow scheduling management, and supply-chain tracking, in a manner that is compliant with 21 CFR Part 11 [13]. This modernization can be supported by a variety of specialized digital tools, including dedicated software for production planning and resource scheduling; comprehensive cell therapy orchestration platforms providing end-to-end workflow management with chain of identity and custody tracking; and enterprise-level eQMS for overseeing documentation, deviations, and change control. When combined with digital interconnectivity of manufacturing equipment, these digital workflow management solutions enable facilities to improve productivity, while optimizing facility and resource utilization.

BEST PRACTICES IN REGULATORY ALIGNMENT

Fostering a collaborative and transparent relationship with regulatory agencies is another key de-risking strategy to streamline progress towards commercialization: leveraging early engagement opportunities such as the FDA's INTERACT meetings (USA), the EMA's Innovation Task Force briefings and Scientific Advice procedures (EU), MHRA's ILAP (UK) and PMDA Sakigake (Japan). Such discussions allow for early feedback on CMC strategy, proposed analytical panel, or overall development plan, helping to align expectations and build regulatory confidence in the program.

CONCLUSION

A phase-appropriate approach for cell therapy manufacturing is essential, but commercial foresight from day one greatly increases the odds of success. Early adoption of enabling technologies, QbD

methodology, automation, digitalization, and CDMO partnerships can ensure that a cell therapy program moves efficiently from preclinical, early-stage trials through to commercial manufacturing, minimizing the risk of costly bottlenecks or late-stage redesigns. Embedding these essential best practices strategically into phase appropriate development will improve the likelihood that cell therapy pipelines achieve both timely patient access and sustainable scalability for commercial success.

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BIOGRAPHIES

Ashwin Srinivasan Kumar is a translational scientist with 8 years of experience focused on advancing cell therapies from the lab to the clinic. In his current role at A*STAR's Process Accelerator for Cell Therapy Manufacturing, he is responsible for developing robust manufacturing processes for novel immunotherapies. Dr Kumar's research leverages state of the art technologies to dissect the complex tumor immune microenvironment. His technical expertise includes high-throughput screening, single-cell genomics, advanced bio-imaging, computational modeling, and finite element analyses to identify novel therapeutic targets and biomarkers. He has a proven track record of translating complex biological questions into robust, data-driven experimental designs, aiming to bridge the gap between discovery and clinical application. His current work builds on his postdoctoral research at Harvard Medical School and the Broad Institute, which focused on translating novel immunotherapies for pediatric medulloblastoma. Dr Kumar earned his PhD in Medical Engineering and Medical Physics from MIT, Cambridge, MA, USA and a BEng from Imperial College London, UK. He has authored multiple peer-reviewed publications in leading scientific journals.

Jaichandran Sivalingam is a Principal Scientist and Group Leader for the Process Accelerator for Cell Therapy Manufacturing (PACTMAN) group at Bioprocessing Technology Institute, A*STAR. A molecular biologist by training with over 20 years of research and industry experience in cell and gene therapy development. He was previously involved in pre-clinical cell therapy pipeline development for diabetes and hemophilia A, at the National Cancer Centre, Singapore. His expertise in cell therapy development includes gene engineering and establishing scalable manufacturing of induced pluripotent stem cells derived hematopoietic stem cells and universal red blood cells for transfusion therapy at BTI (A*STAR). He was part of the Process, Science and Technology (PSAT) team involved in CAR-T pipeline development at Tessa Therapeutics, formerly a late-stage clinical company involved in immune-oncology pipeline development. He currently leads the PACTMAN group, A*STAR's cell and gene therapy initiative, to provide pre-GMP Process and Analytical Development support for cell therapy asset developers from academia, national healthcare groups and local biotech companies and serves as a review editor on the editorial board of Cancer Immunity and Immunotherapy.

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Sabry Hamza is a seasoned biotechnology leader with over 20 years of experience spanning academia and industry, specializing in virology, oncology, immunology, neuroscience, and cell biology. He currently serves as Head of Process Development and Analytical Sciences at Tikva Allocell, where he leads the strategic design, development, and optimization of CAR-T cell manufacturing processes and analytical methods for cell and gene therapies. Dr Hamza has a proven track record of guiding programs from early discovery through clinical development, including drafting Chemistry, Manufacturing, and Controls (CMC) sections for IND and global regulatory submissions. His expertise includes establishing scalable manufacturing workflows, developing lot release assays, and ensuring regulatory compliance with FDA and EMA guidelines. Prior to his current role, he held senior positions at Tessa Therapeutics and Merck Sharp & Dohme, where he contributed to innovative platforms in cell therapy and translational medicine research. Dr Hamza earned his PhD in Comparative Pathology

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

Time for the FDA and industry–academia partnerships to invest in fully automated, quality-controlled, scale-out of autologous cell and gene therapies

Krishnendu Roy

VIEWPOINT

"Patients have waited too long already—it is time we fulfill our obligation to them."

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The field of cell and gene therapy (CGT) has achieved transformative successes in several hematological cancers and is now making significant strides in solid tumors and autoimmune diseases. Eight years have passed since the approval of the first CAR-T cell product, and since then, billions have been invested by big pharma, private investors, and CDMOs. Yet, by some estimates, less than 50,000 patients have been treated with CAR-T cells worldwide, a number, although formidable and practice-altering, still falls woefully short of the global patient population that needs it and, frankly, deserves to have access to. As we break into the solid tumor and the autoimmune spaces with a portfolio of autologous therapeutic cells, the demand will be exponentially larger. The industry (and government) needs to employ a radically different strategy than what we have done so far, so that we can deliver the promise

to many more patients, at a significantly lower infrastructural and healthcare cost, and within a much faster timeframe, without the long turnaround delays that have plagued the field. Yes, *in vivo* cell therapy through direct gene transfer using lipid nanoparticles (LNPs), such as those being invested heavily by some major pharmaceutical companies recently, can address many of these issues. However, those 'drug-like', potentially short-lived therapy models remain far from proven, especially in terms of their durability and safety in large patient cohorts.

The explosion of CGT CDMOs, despite challenges of process and analytical tech-transfer even within a single organization, is not a long-term solution to the huge demand and supply mismatch of CGTs, especially in resource constrained areas. What is instead needed is a concerted, global effort between industry and academia, partnered with the US FDA and other international regulatory agencies, to enable end-to-end automated, quality-enabled, small-footprint, scale-out manufacturing solutions at or near (local or regional) the patients. This can avoid the expensive and complex logistical and cold-chain challenges, as well as the challenges of complicated tech transfer and the substantial infrastructural investment in manufacturing required in current centralized models, which are often impossible for small and medium-sized companies to overcome. To be clear, I am not advocating against centralized manufacturing; rather I am advocating that automation and quality-enabled scale-out need to be a significant additional part of the solution.

The challenges, though, are twofold: First, how does one ensure that every batch manufactured at every bedside or local/regional facility passes rigorous quality standards, reproducibly, that the FDA will be comfortable with given their long history of relying on a centralized quality assurance models; and second, how do we design and develop modular, plug-and-play automation units with built-in quality assessment and process controls, that can be used for a variety of workflows—starting from the apheresis step and ending in a ready-to-deliver formulation of therapeutic cells of the correct phenotype, number, and quality?

The former is where we need the FDA to collaborate and fund active research and development between industry and academia, and where the National Institute of Standards and Technology (NIST) and organizations like the Standard Coordinating Body (SCB) need to play a project-based, time-limited (not open-ended) accelerator role. What should we measure, in or at line, in real-time, to ensure quality and adjust processes to achieve a set phenotype of the finished product that is predictive of function in a given group of patients? We still have not come close to answering this question, despite several academic groups attempting to do so. And, frankly, the National Institutes of Health (NIH) and the National Science Foundation (NSF) cannot simply be bystanders in this effort, saying that manufacturing is not their 'domain'. There is still a lot of fundamental biology and engineering that needs to happen for us to understand precisely how a system of process parameters may drive a population of heterogeneous cells into a narrow quality phenotype and how cells with dynamically changing environmentally driven properties behave inside a given patient—a problem well within their 'domain'.

The second challenge can only be solved through a combination of engineering and biology expertise. Despite many inroads into benchtop systems driving CGT automation in recent years, there is still a tremendous amount left to be achieved. To make the low-cost, quality-enabled scaling-out vision come true, we must be able to start with the 'raw' patient sample that can be 'fed' into an automation system without prior manual processing, which can then

undergo fully automated cell isolation, purification, genetic or phenotypic manipulation, culture, expansion, end-process purification, and processed into a ready-to-deliver formulation, all while ensuring quality measurements (preferably non-destructive and real-time) and feedback control at each step. This is a very tall order for a 'living' product whose properties change with each of those manipulations. However, given the tremendous strides we have made in biology, robotics, automation, sensors, process control, and artificial intelligence over the past decade, we can achieve this—but only if there is a concerted and directed investment by government, industry, and academia—with urgency and focus, akin to the 'Manhattan Project'. If we are to deliver CGT products on demand to every corner of the population who need them, we, as a society, must make this commitment.

One might argue that the government should not invest in this vision—it is the industry's domain. I am sure we could have said the same thing for nuclear energy, the internet, radar, autonomous cars, transportation systems, defense technologies, vaccines, life-saving drugs, and many more transformative technologies—but we did not, because there is a fundamental societal benefit tied to all of these, as it is to the success of advanced CGT. That makes co-investment by both the public and private sectors essential. Patients have waited too long already—it is time we fulfill our obligation to them.

BIOGRAPHY

Krishnendu (Krish) Roy received his BTech from the Indian Institute of Technology (IIT), Kharagpur, India, his MS from Boston University, Boston, MA, USA and his PhD in Biomedical Engineering from Johns Hopkins University, Baltimore, MD, USA. After 2 years in the biotech industry, Dr Roy joined the Biomedical Engineering Faculty at the University of Texas at Austin in 2002, eventually becoming a Professor and a Fellow of the Cockrell Chair in Engineering Excellence. In 2013, he moved to Georgia Tech, where he was most recently Regents Professor and the Robert A Milton Endowed Chair in Biomedical Engineering. He also served as Director of three centers—the NSF Engineering Research Center (ERC) for Cell Manufacturing Technologies (CMaT), the Marcus Center for Therapeutic Cell Characterization and Manufacturing (MC3M), and the Center for ImmunoEngineering. In August 2023, Dr Roy joined Vanderbilt University as the Bruce and Bridgitt Evans Dean of Engineering and a University Distinguished Professor of Biomedical Engineering, as well as Pathology, Microbiology, and Immunology. Dr Roy's research interests include the scalable manufacturing of immune cells, immuneengineering, and controlled drug and vaccine delivery technologies, with a focus on biomaterials. In recognition of his seminal contributions, Dr Roy has been elected Fellow of the American Institute for Medical and Biological Engineering (AIMBE), the Biomedical Engineering Society (BMES), and the Controlled Release Society (CRS). He has received numerous awards and honors, including the Clemson Award for Basic Research from the Society for Biomaterials, the Industry Growth Award from Georgia Bio, and Young Investigator Awards from both the Controlled Release Society (CRS) and the Society for Biomaterials (SFB). He has also received the NSF CAREER award, among others. He has also received the Best Teacher Award from the Biomedical Engineering Students at UT-Austin and the Best Advisor Award from Bioengineering students at Georgia Tech. Dr Roy serves on the Editorial Boards of the *Journal of Immunology* and *Regenerative Medicine*, the *European Journal of Pharmaceutics and Biopharmaceutics*, and the *Journal of Advanced Biomanufacturing and Bioprocessing*, and from 2006 to 2025 served on the Editorial Board

of the *Journal of Controlled Release*. He also serves as a board member of the Standards Coordinating Body for Regenerative Medicine and several other academic and industry advisory boards. He also co-chairs the Forum on Regenerative Medicine of the National Academies of Science, Engineering, and Medicine (NASEM).

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

SCALE-UP/-OUT OF CELL AND GENE THERAPY MANUFACTURING

SPOTLIGHT

EVENT PREVIEW

Biologics CDMO Europe 2025

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As part of our ongoing coverage of key gatherings in life sciences, BioInsights presents a preview of Biologics CDMO Europe 2025. Scheduled for November 19–20, 2025, in Munich, Germany, this summit will unite up to 300 senior manufacturing and external supply-chain experts from across Europe. Focusing on agile, tech-enabled biologics manufacturing, regulatory alignment, and strategic CDMO partnerships, the agenda features off-the-record case studies, executive roundtables, and deep-dive sessions.

OUTSOURCING STRATEGY AND CDMO PARTNER EVALUATION

A strategic panel including Suyamburam Sathasivam (Associate Vice President, SUN PHARMA), Ulrich Rümenapp (Head of Launch Preparation and Coordination, Bayer), and Daniel Hurni (Former Director of Manufacturing Network Strategy and Business Intelligence, Bristol Myers Squibb) will discuss outsourcing trends toward 2030. Additionally, Christopher Pawlak (External manufacturing Lead, Bayer) will outline practical tools for CDMO partner evaluation, while Andreas Schaaf (Managing Director/CSO, Eleva) will highlight innovations in biomanufacturing technologies. Key sessions will also explore risk allocation in CDMO agreements and resilient partnership models, setting a collaborative tone for navigating Europe's evolving biologics landscape.

TECH TRANSFER AND GLOBAL REGULATORY HARMONIZATION

The summit will also focus on tech transfer and regulatory compliance for advanced therapies. Christian Simon (Head of Technical Transfer External Manufacturing, Sanofi) will explore how AI-driven predictive maintenance can reduce downtime and improve equipment performance. Jenny Prange (CTO, Muvon Therapeutics) will present strategies for navigating tech transfer in regenerative therapies. Furthermore, a panel on global regulatory harmonization will follow, featuring Pavan Beleyur Narayanaswamy

(Head of CMC and Regulatory Affairs, AATec Medical) and Eoin McGrath (Executive Director, ICCBBA).

COST OPTIMIZATION AND EVOLVING CONTRACT MODELS

Ulrich Rümenapp (Head of Launch Preparation and Coordination, Bayer) will address strategic approaches to outsourcing CMC development and manufacturing, including IP protection and building effective CDMO partnerships. Giulio Cavalli (Principal Lead, External Manufacturing, Johnson & Johnson Innovative Medicine) will share best practices for managing cross-border tech transfers in a globalized production landscape. The summit will also include a panel discussion on the evolution of contract models in biomanufacturing, featuring Ralf Huss (Managing Director, Biom Biotech Cluster) and Chris Baldwin (Vice President, Manufacturing and Supply, Resolution Therapeutics), who will explore shifting trends and collaborative opportunities in outsourcing agreements.

CELL & GENE THERAPY INSIGHTS

ANALYTICS

REVIEW

From bench to bedside: navigating bioanalytical method development and validation for clinical efficacy and regulatory compliance

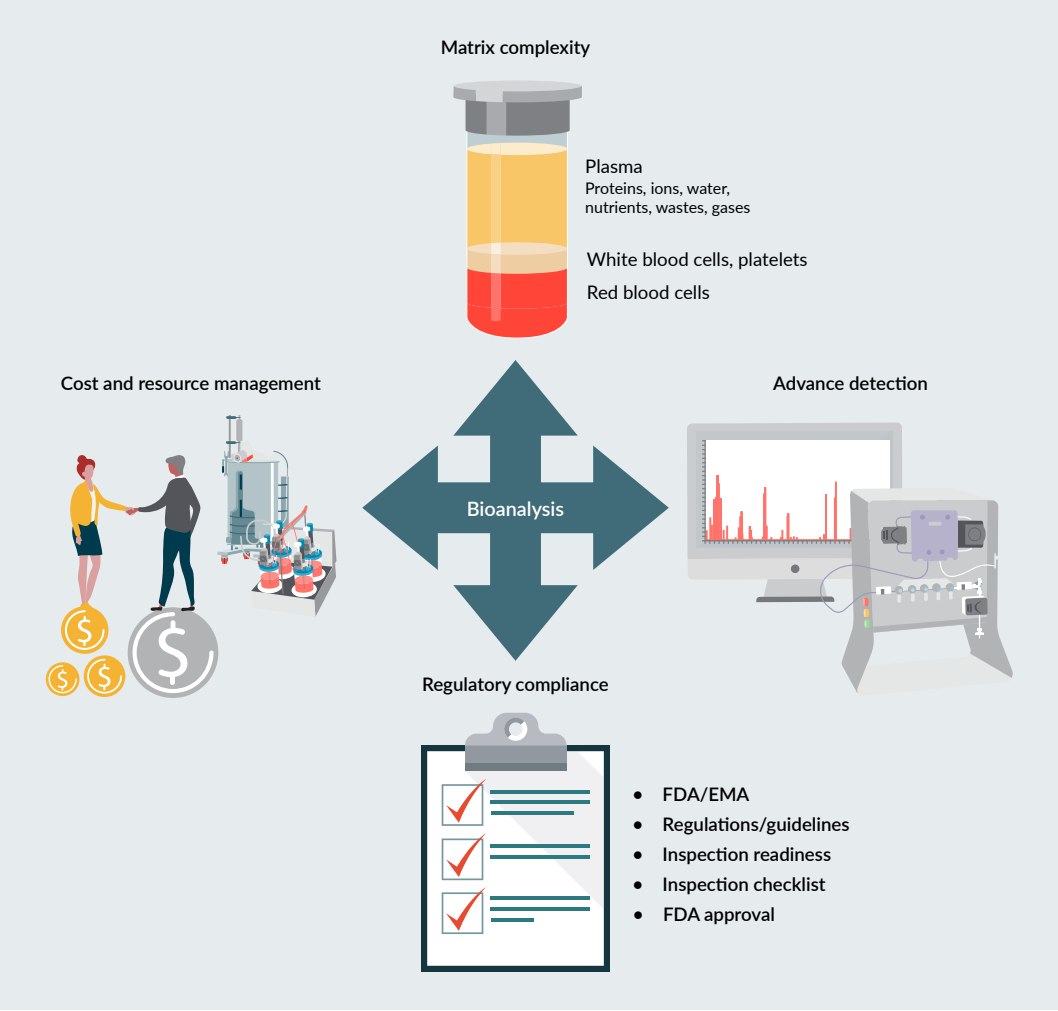
Preeti Misra

Bioanalytical methods are indispensable in pharmaceutical development, serving as the backbone for ensuring clinical efficacy and regulatory compliance. The development and validation of these methods face numerous challenges for improved clarity and flow, as recommended. Biological matrices such as blood, plasma, urine, and tissues often contain endogenous substances that interfere with analyte detection, complicating efforts to achieve accuracy and reliability. These challenges highlight the need for innovative strategies to optimize bioanalytical methods for both clinical and regulatory success.

Biological matrices, ranging from those encountered in small molecule studies to complex biologics, including cell and gene therapies, present inherent complexities due to endogenous biomolecules that can interfere with accurate detection and quantification of analytes, such as therapeutic vectors or transgene products. Effective sample preparation, matrix-matched calibration, and advanced detection techniques are essential to mitigate matrix effects and enhance sensitivity. Achieving high selectivity is especially critical in early-phase clinical studies and biomarker analysis. Advanced instrumentation, derivatization techniques, and multiple reaction monitoring (MRM) are pivotal for precise quantification and reliable results. Adherence to stringent guidelines from regulatory bodies such as the US FDA and EMA requires thorough method validation, meticulous documentation, and consistent implementation of regulatory standards. Stability studies are essential to address challenges related to analyte degradation. Techniques such as stabilization methods and rapid processing are critical to maintaining analyte integrity throughout the analytical process. Developing bioanalytical methods is resource-intensive, requiring efficient cost management, strategic planning, and where appropriate, collaboration with academic institutions and contract research organizations (CROs) to optimize processes.

Bioanalytical methods are essential for advancing pharmaceutical development from bench to bedside, providing the accuracy and reliability needed to support clinical efficacy and regulatory compliance. Key challenges—including matrix complexity, limited sensitivity,

analyte instability, and stringent regulatory expectations—demand robust and well-validated methods. To address these issues, laboratories are increasingly leveraging advanced strategies such as optimized sample preparation techniques, matrix-matched calibration, and MRM. These approaches help mitigate matrix effects, improve detection sensitivity, and ensure analyte stability across diverse biological matrices. By integrating such practical and innovative solutions, bioanalytical workflows can achieve greater efficiency, accuracy, and regulatory alignment, ultimately facilitating successful clinical development and approval.



Key challenges and strategic solutions in bioanalytical method development. This graphical abstract summarizes key challenges in bioanalytical method development—such as matrix complexity, sensitivity, regulatory compliance, and stability—and showcases strategic solutions like advanced sample prep, matrix-matched calibration, MRM, stability studies, and automation to enhance accuracy, reliability, and compliance.

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INTRODUCTION

Bioanalytical method development plays a pivotal role in the development and therapeutic monitoring of biologics, including monoclonal antibodies, recombinant proteins, antibody–drug conjugates, and cell and gene therapies. These methods are essential for determining the concentration of the drug and its metabolites and

biomarkers in physiological fluids, such as blood, serum, plasma, urine, and cerebrospinal fluid; tissue, such as skin; and tumor biopsies. Bioanalytical data related to the pharmacokinetic (PK), pharmacodynamic (PD), and toxicokinetic profiles of a drug are precise and highly useful and recorded for therapeutic drug monitoring (TDM) [1,2] which is essential for evaluating the drug's safety and efficacy, as well as guiding regulatory decisions. Significant advancements in bioanalytical technologies have revolutionized drug development and clinical research. High-resolution mass spectrometry (HRMS) and ultra-high-performance liquid chromatography (UHPLC) have greatly enhanced the sensitivity and selectivity of analyte detection [3]. Automated sample preparation and data analysis systems have increased throughput and reproducibility, reducing labor costs and human error [4]. Additionally, advanced techniques such as MRM in tandem mass spectrometry (MS/MS) allow for precise quantification of target analytes amidst complex biological matrices [5]. Despite the significant advancements in bioanalytical technologies, numerous challenges persist in method development, validation, and application [6,7]. This review discusses the key challenges in bioanalytical method development and potential strategies to address them.

Successful drug development and drug safety depend on numerous analytical testing processes at several steps during the development pipeline, and during ongoing characterization of commercial drugs. It is essential that the industry and patients can rely on the accuracy and reproducibility of this analytical testing. Over the years we have seen continual improvements in the capabilities of analytical technologies, driven by the highly competitive and strictly regulated nature of the pharmaceuticals industry. Due to innovative techniques the bioanalytical testing services market is expanding rapidly, driven by the increasing demand for precise, reliable analytical methods in drug development and clinical research. The global bioanalytical testing services market size was US$4.78 billion in 2023, accounted for US$5.22 billion in 2024, and is expected to reach around US$12.59 billion by 2034, expanding at a compound annual growth rate (CAGR) of 9.2% from 2024 to 2034 [8]. As the complexity of new therapeutic modalities grows, bioanalytical testing services address critical challenges such as matrix effects, sensitivity, selectivity, regulatory compliance, and analyte stability. The market's growth reflects the need for advanced technologies, such as HRMS, UHPLC, and automated systems, which enhance method robustness and efficiency. By outsourcing to specialized bioanalytical testing service providers, pharmaceutical companies can mitigate costs, streamline development timelines, and ensure adherence to stringent regulatory standards, ultimately improving drug safety and efficacy [9,10]. During recent years, there have been a number of innovative developments in instrumentation engineering, as well as in method development, which are allowing scientists to explore novel therapeutic molecules and increasingly complex compounds. In the future these developments are likely to require even more diverse analytical methods, with continual improvements in speed, selectivity, and accuracy.

BIOANALYSIS IN SMALL MOLECULE DRUGS

Small molecules, defined as organic compounds with low molecular weight (below 900 Daltons), are essential in biology and medicine, encompassing drugs, metabolites, hormones, neurotransmitters, and other critical health-related molecules. Accurate measurement of these molecules in biological samples is vital for drug development, as it informs the pharmacokinetics of drug candidates, helping researchers

optimize dosing regimens and assess safety [11]. Additionally, in disease diagnosis, biomarker discovery and quantification are crucial for early detection, prognosis, and monitoring therapeutic responses. In metabolomics, small molecule profiling offers insights into metabolic pathways and their alterations in various diseases [12]. Small molecule bioanalysis is a multidisciplinary field with wide-ranging applications in healthcare and research. It is a complex process that requires an agile approach, and researchers often face several challenges when developing and validating bioanalytical methods. Challenges include addressing matrix effects, where endogenous substances interfere with detection, and ensuring efficient sample preparation and analyte stability [13]. Accurate calibration and method validation are crucial, adhering to regulatory guidelines like those from the FDA and ICH [14]. Emerging trends include advancements in automation, high-throughput screening, and lab-on-a-chip technologies, which enhance efficiency and precision. These innovations not only improve the speed and scalability of analyses but also help to address key challenges such as matrix complexity, sensitivity limitations, and analyte stability. For example, high-resolution mass spectrometry and microfluidic platforms enable more selective detection and streamlined sample preparation, reducing interference from biological matrices. Despite the inherent complexities of small molecule bioanalysis, these advancements in bioanalytical techniques continue to drive improvements in drug development and clinical testing, facilitating reliable and robust analysis of small molecules in biological matrices. In this field, researchers and analysts must stay informed about emerging technologies and adhere to rigorous validation and regulatory standards to ensure the reliability and accuracy of their analyses. Navigating the world of small molecule bioanalysis is an ongoing journey, with each advancement bringing us closer to a deeper understanding of biology and improved healthcare outcomes.

BIOANALYSIS IN LARGE MOLECULE DRUGS

Novel target identification technologies, combined with emerging new drug modalities beyond classic small molecules, monoclonal antibodies, antibody drug conjugates, and cell and gene therapies– have significantly expanded the range of options to unlock new solutions for unmet medical needs [15]. Large molecules encompass a broad spectrum of entities that vary significantly in size and complexity—from relatively small protein therapeutics (e.g., insulin, ~6 kDa) to much larger structures like lipid nanoparticles (LNPs), which can exceed hundreds of kilodaltons. These size differences can influence the selection of analytical techniques, as larger entities may require alternative separation methods (e.g., field-flow fractionation or size-exclusion chromatography) and more specialized detection systems. Additionally, the physicochemical diversity among large molecules necessitates tailored sample preparation strategies and instrument configurations to ensure reliable quantification and characterization. Assessing the safety and efficacy of new therapeutic modalities requires understanding of their pharmacokinetic (PK) and toxicokinetic (TK) profile. Consequently, innovative bioanalytical strategies are essential for accurately measuring parent drugs, metabolites, and potential immunogenic responses. Bioanalysis of large molecule drugs, including biologics such as monoclonal antibodies, antibody drug conjugates, proteins, peptides, and for non-protein drugs like RNA, cell, and gene therapies involves complex techniques to measure and characterize these molecules in biological matrices [16,17]. Technology platforms other than liquid chromatography coupled with mass spectroscopy

(LC-MS) and ligand binding assay (LBA) [18,19], such as quantitative polymerase chain reaction (qPCR), sequencing, hybrid LBA, enzyme-linked immunosorbent assays (ELISA), surface plasmon resonance (SPR) or flow cytometry, are necessary to measure the molecular or cellular analysis of drug [20–23]. Each of these techniques offers unique advantages for quantifying large molecules and understanding their pharmacokinetics and immunogenicity for instance, qPCR is preferred for sensitive detection of nucleic acids, whereas SPR excels in real-time monitoring of molecular interactions. The primary challenges in this field include managing assay specificity and sensitivity, dealing with complex matrix effects, and ensuring stability and accurate quantification of large molecules, which can be affected by factors such as glycosylation and protein aggregation and can have significant implications for therapeutic efficacy and safety. To mitigate these issues, rigorous method validation and strict adherence to regulatory guidelines are paramount. Emerging technologies, including biosensor platforms and advanced mass spectrometry techniques, are continually enhancing the precision, throughput, and robustness of large molecule bioanalysis. These advancements facilitate more reliable and efficient characterization of complex biologics, supporting the development of safer and more effective therapeutics.

The primary challenges in the development of bioanalysis methods discussed above are outlined below in Table 1, which outlines assay specificity and sensitivity, managing matrix effects, ensuring the stability and accurate quantification of

TABLE 1

Key analytical challenges in bioanalytical method development and their relevance to ICH Q2 (R2) validation guidelines.

Challenges	Description	ICH Q2 (R2) relevance
Sensitivity and specificity	Sensitivity is crucial for detecting low concentrations; specificity ensures distinction from endogenous compounds	Addressed (specificity, detection limit)
Matrix effects	Endogenous substances in biological matrices can interfere, causing ion suppression or enhancement	Partially addressed (robustness)
Extraction efficiency	Efficient extraction and preparation methods are needed to handle the complexity of biological samples and ensure consistent recovery and reproducibility	Addressed (accuracy, recovery)
Stability of analytes	Analytes can degrade due to chemical instability, enzymatic activity, or environmental conditions; large molecules can degrade due to factors like glycosylation and protein aggregation during sample collection, storage, and analysis, leading to inaccuracies	Addressed (stability)
Calibration and quantitation	Accurate calibration curves are necessary for reliable quantification; standards must be validated for linearity and precision	Addressed (linearity, quantitation)
Regulatory compliance	Methods must adhere to stringent guidelines set by regulatory agencies such as FDA, EMA, and ICH	Covered
Technological advancements	Integrating new technologies such as biosensor platforms and HRMS, automation, and microfluidics poses challenges into existing workflows while maintaining compliance with regulatory standards	Not directly covered
Inter-individual variability	Variability in biological matrices due to genetic, environmental, and lifestyle factors affects method performance	Partially addressed (robustness, reproducibility)

The table distinguishes between challenges fully addressed by ICH Q2 (R2) and those only partially covered or outside its direct scope, highlighting the need for both regulatory compliance and practical considerations in method design.

analytes, dealing with complex sample preparation, method validation, regulatory compliance, and integrating emerging technologies.

The primary objective of bioanalytical method development is to delineate the procedures and operating conditions under which a method can effectively extract, identify, and quantify the analyte(s) of interest and/or their metabolites for the intended application. Given the distinct physicochemical properties of small and large molecule drugs, different strategies must be employed for the development of an effective and efficient bioanalytical method. Following section provides an overview of a few challenges in sample preparation strategies, analytical platforms, procedures for achieving high throughput, regulatory guidelines, stability of analytes and cost management, in the bioanalysis of both small and large molecule drugs.

MATRIX COMPLEXITY IN BIOANALYSIS

Problem: endogenous interference in biological matrices

Biological matrices such as blood, plasma, urine, and tissues are inherently complex and present significant challenges in bioanalytical method development. These matrices contain a wide array of endogenous substances such as proteins, lipids, salts, and metabolites, which can interfere with the analyte of interest. This interference, known as matrix effects, can significantly impact the accuracy, precision, and sensitivity of bioanalytical methods. Matrix effects occur when co-eluting substances alter the ionization efficiency of the analyte during mass spectrometric analysis, leading to signal suppression or enhancement [24].

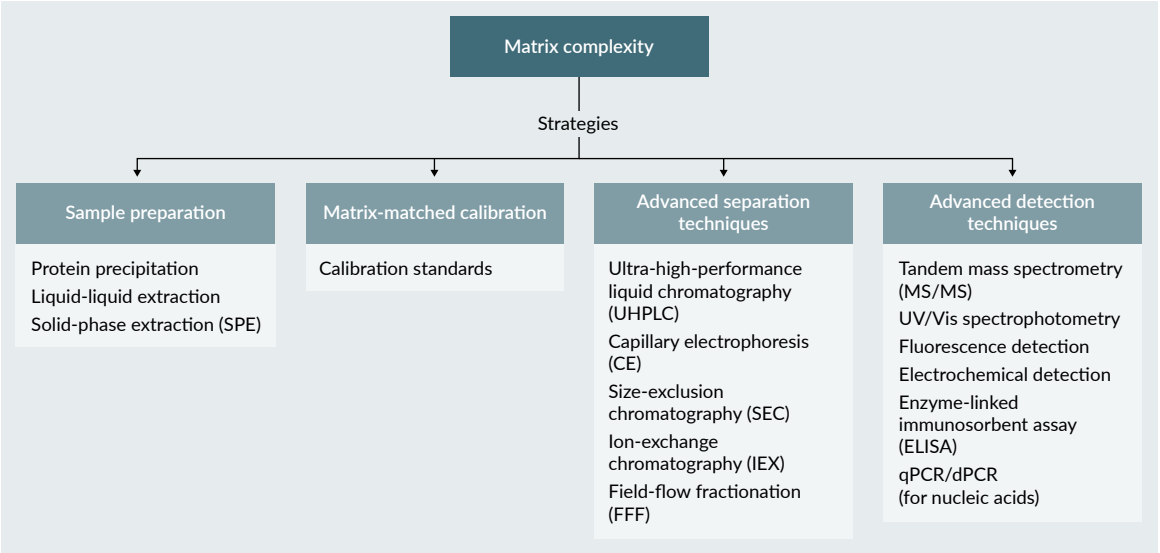
Impact: reduced assay performance and data variability matrix effects can result in inaccurate quantification and poor reproducibility of analytical results—particularly for low-abundance analytes or those requiring high selectivity. In early-phase clinical studies, where precision is critical, even minor variability due to matrix interference can significantly affect pharmacokinetic interpretation and regulatory acceptability. Furthermore, inter-individual and species-based matrix variability adds another layer of complexity in translational studies.

Solution: sample preparation and advanced detection addressing matrix complexity is crucial for developing robust and reliable bioanalytical methods [25]. Strategies to address matrix complexity include effective sample preparation, using matrix-matched calibration and employing advanced detection techniques (Figure 1).

Effective sample preparation is essential to minimize matrix effects and improve the performance of bioanalytical methods [26,27]. Strategies often differ depending on whether the analyte is a small molecule or a large biomolecule. For small molecules, techniques such as protein precipitation, liquid-liquid extraction (LLE), and solid-phase extraction (SPE) are commonly used to remove proteins and other interfering substances from biological matrices like plasma or serum. Protein precipitation involves adding organic solvents (e.g., acetonitrile, methanol) to precipitate endogenous proteins, which are then removed by centrifugation. While this method is rapid and amenable to high-throughput workflows, it may not eliminate all matrix interferences. LLE separates analytes based on their solubility in immiscible solvents, offering effective cleanup but requiring manual steps and longer processing times. SPE uses solid adsorbents to retain analytes while washing away contaminants and provides high selectivity when properly optimized. For large biomolecules such as proteins, peptides, or LNPs, sample preparation techniques may instead focus on isolating the target molecule rather than removing it. In such cases, buffer exchange,

FIGURE 1

Strategies to address matrix complexity.



ultrafiltration, or affinity purification may be employed to reduce matrix complexity while preserving the analyte. Additionally, simple dilution with appropriate buffers is sometimes sufficient to mitigate matrix effects, particularly when using highly sensitive detection platforms. Clarifying the specific goals of each technique, whether to remove matrix components or to isolate the analyte, is crucial in method development for diverse therapeutic modalities [28,29]. Using matrix-matched calibration standards involves preparing calibration standards in the same biological matrix as the samples. This approach accounts for matrix effects by ensuring that the calibration curve reflects the same matrix environment as the unknown samples, leading to more accurate quantification [30].

Employing advanced detection techniques can enhance the specificity and sensitivity of bioanalytical methods [31,32], thereby mitigating matrix effects: MS/MS provides high specificity by detecting unique fragmentation patterns of analytes. MRM mode in MS/MS can selectively quantify target analytes, reducing interference from co-eluting substances. HRMS offers superior mass accuracy and resolution, UHPLC improves chromatographic separation, reducing co-elution of interfering substances with the analyte. This enhances the method's sensitivity and reduces matrix effects.

Sensitivity and selectivity

In bioanalytical method development, sensitivity and selectivity are critical parameters that determine the method's capability to detect and accurately quantify low concentrations of analytes in complex biological matrices. High sensitivity ensures that even trace levels of analytes can be detected, which is essential in early-phase clinical studies and biomarker analysis. Selectivity, on the other hand, ensures that the method can accurately distinguish the analyte from other similar compounds and endogenous substances present in the matrix. To enhance these parameters, several advanced techniques are employed. HRMS improves both sensitivity and selectivity through high mass accuracy and resolution, making it valuable for identifying drug metabolites and low-abundance

biomarkers [33]. UHPLC provides superior chromatographic separation, improving resolution and reducing co-elution effects—especially beneficial in analyzing complex matrices like plasma or tissue homogenates [34]. Chemical derivatization involves modifying the chemical structure of the analyte to improve its detectability. This is particularly helpful in enhancing the detectability of poorly ionizing compounds in LC-MS/MS assays [35]. It can also improve the chromatographic behavior of analytes, leading to better separation and reduced matrix effects [36]. MRM is a mode of MS/MS that enhances selectivity and sensitivity by monitoring specific precursor ions to produce ion transitions. MRM enhances selectivity by monitoring specific ion transitions unique to the target analyte, reducing the interference from co-eluting compounds and matrix components [37]. MRM mode provides enhanced sensitivity as it focuses the detection on specific ion transitions, leading to better signal-to-noise ratios and lower limit of quantitation (LOQ) and limits of detection (LOD) [38]. This heightened sensitivity is particularly critical in quantifying trace-level analytes in complex biological matrices, where achieving a low LOD ensures accurate detection of compounds present in minute concentrations, supporting applications such as biomarker discovery, residual drug monitoring, and early-phase clinical studies. Improving sensitivity and selectivity is not only essential for robust quantification but also has direct implications for regulatory compliance. Methods that can reliably detect and differentiate analytes in complex matrices are more likely to meet FDA and ICH bioanalytical method validation guidelines, thereby reducing regulatory risks and facilitating clinical decision-making.

STABILITY OF ANALYTES

Bioanalytical testing involves multiple steps (Figure 2) including sample collection, processing, storage, and extraction, as well as analyte detection and quantitation.

The stability of analytes in biological samples is a critical factor that impacts the accuracy and reliability of bioanalytical methods. Analytes can degrade due to various factors, including enzymatic activity, chemical reactions, and environmental influences such as temperature and light. Ensuring analyte stability throughout the entire analytical process, from sample collection to analysis, is essential for obtaining accurate and reproducible results. Conducting stability studies under various conditions is a fundamental step in assessing the stability of analytes in biological matrices. These studies help determine the conditions under which the analytes remain stable and provide guidance for sample handling and storage. Short-term stability evaluates the stability of analytes at room temperature for a specified period to simulate conditions during sample processing and handling [39]. Long-term Stability assesses the stability of analytes when stored at low temperatures (e.g., −20 °C or −80 °C) over an extended period to ensure stability during long-term storage [40]. Freeze–thaw stability tests the stability of analytes through multiple freeze-thaw cycles to determine their stability during repeated freezing and thawing processes [41]. Employing stabilization techniques can enhance the stability of analytes in biological samples, thereby reducing the risk of degradation during sample handling and storage. Add stabilizing agents to the sample to inhibit enzymatic activity or chemical reactions that may cause degradation. For example, protease inhibitors can be added to plasma samples to prevent protein degradation [42]. Adjust the pH of the sample to a level that minimizes analyte degradation. For example, acidic or basic conditions can stabilize certain compounds by preventing hydrolysis or oxidation [43]. Store samples under controlled conditions (e.g., low temperatures, protection from light) to prevent

FIGURE 2

Steps involved in bioanalytical analysis.

1. **Sample Collection** should be performed using sterile techniques in a minimally invasive manner, using the smallest amount necessary for reliable quantification.
2. **Sample Processing** involves such as centrifugation followed by immediate freezing, appropriate aliquoting and preservation methods, such as snap freezing or chemical stabilization, Accurate labeling and documentation to ensure traceability and integrity throughout the processing workflow.
3. **Sample Storage** should be well defined and validated including temperatures, storage duration etc. Use clearly labeled, airtight containers to prevent contamination and degradation. Regularly monitor storage conditions to ensure consistent temperature and integrity. Implement a reliable inventory system to track sample locations and storage durations.
4. **Sample Extraction** is a technique to clean up a sample before analysis and/or to concen trate a sample to improve its detection. It may be performed using protein precipitation, solid phase extraction, liquid liquid extraction, solid phase microextraction, matrix solid phase dispersion, column switching or supercritical fluid extraction.
5. **Sample Detection** The detector of choice is a mass spectrometer. principle technique used in quantitative bioanalysis is high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) techniques.

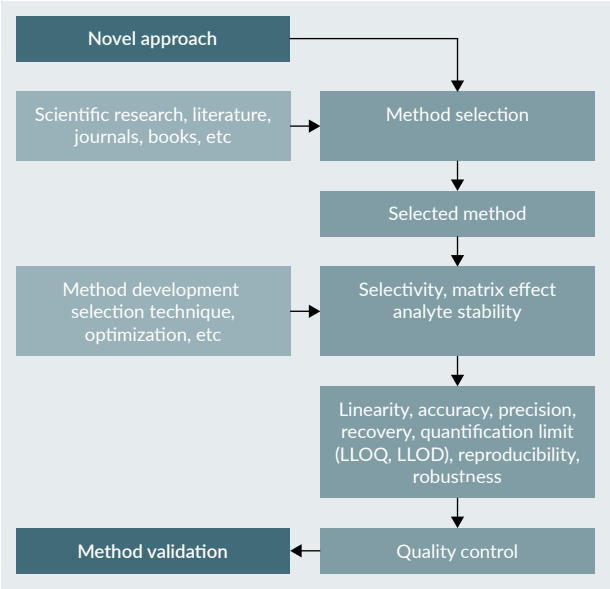
degradation. For example, light-sensitive compounds should be stored in amber vials to protect them from photodegradation [44]. Minimizing the time between sample collection and analysis is crucial for reducing the risk of analyte degradation. Rapid processing helps preserve the integrity of the analyte by minimizing exposure to conditions that may cause degradation. Immediately process and stabilize samples after collection to prevent enzymatic or chemical degradation. For example, blood samples should be centrifuged and plasma separated promptly to prevent hemolysis and degradation of labile compounds [42]. Prioritize the analysis of samples to reduce the storage time and potential degradation. Implementing streamlined workflows and automated sample handling systems can facilitate rapid processing [45]. Ensuring the stability of analytes in biological samples is crucial for the accuracy and reliability of bioanalytical methods. Conducting comprehensive stability studies, implementing effective stabilization techniques, and minimizing the time between sample collection and analysis are critical strategies for preserving analyte integrity. These practices not only mitigate degradation risks but also enhance the robustness, reproducibility, and regulatory acceptability of bioanalytical methods—ensuring reliable data to support clinical decision-making and drug approval processes.

REGULATORY COMPLIANCE

Ensuring regulatory compliance is a fundamental aspect of bioanalytical method development. However, like any production process, bioanalytical methods undergo a lifecycle—they are developed, evolve, and eventually become obsolete. To fully understand the significance of validation in the life of a bioanalytical technique, it is essential to describe its lifecycle from the time it is selected until it is ultimately retired. The life cycle of bioanalytical method validation, as represented in Figure 3, illustrates that bioanalytical processes are often

FIGURE 3

Bioanalytical method lifecycle.



described as fixed procedures, but in reality, they undergo continuous refinement and adaptation to meet evolving scientific and regulatory demands.

Regulatory agencies like the FDA and EMA provide stringent guidelines to ensure that bioanalytical methods are reliable, reproducible, and robust [46,47]. Compliance with these guidelines is essential for the acceptance of bioanalytical data in regulatory submissions, including new drug applications (NDAs), biologics license applications (BLAs), and clinical study reports.

To ensure regulatory compliance comprehensive method validation is crucial for demonstrating that a bioanalytical method is fit for its intended purpose. Regulatory guidelines outline several key parameters (Table 2) that must be validated.

Detailed documentation and transparent reporting are essential components of regulatory compliance. Accurate and thorough documentation ensures that the method development and validation processes are clearly described and reproducible. This includes detailed reports on the method development process, including rationale for the chosen method, optimization steps, and preliminary validation results. Comprehensive validation reports documenting the validation experiments, data analysis, and conclusions. These reports should include raw data, statistical analysis, and any deviations from standard procedures. Well-documented standard operating procedures (SOPs) for all aspects of the bioanalytical method, including sample preparation, instrument operation, data analysis, and reporting. Strict adherence to regulatory guidelines is essential for ensuring that bioanalytical methods meet the required standards of quality and reliability: The FDA's Bioanalytical Method Validation Guidance for Industry provides comprehensive guidelines on the validation of bioanalytical methods. These guidelines cover all aspects of method validation, including accuracy, precision, specificity, sensitivity, reproducibility, and stability [48]. The EMA's Guideline on Bioanalytical Method Validation outlines the requirements for the validation of bioanalytical methods used in clinical trials and for regulatory submissions in Europe. These guidelines emphasize the importance of robustness, reproducibility, and reliability of bioanalytical methods [49–51].

Regulatory compliance is a critical aspect of bioanalytical method development. Ensuring comprehensive method validation, maintaining detailed documentation and reporting, and adhering to regulatory guidelines are essential strategies for achieving compliance. By following these practices, bioanalytical laboratories can develop robust, reliable, and regulatory-compliant methods, facilitating successful drug development and regulatory approval [52–54].

COST & RESOURCE MANAGEMENT

Developing and validating bioanalytical methods is resource-intensive, often

TABLE 2

Bioanalytical method validation parameters (as outlined in ICH M10 and Q2[R2] Guidelines).

Parameter	Description	Reference
Accuracy	The closeness of the test results to the true value; validation studies should assess the accuracy of the method across the intended range of analyte concentrations	[45]
Precision	The degree of reproducibility of the method under the same conditions over	[46]
Linearity	The ability of the method to produce results that are directly proportional to the concentration of analyte in the sample; a linear calibration curve should be established over the intended range of the method	[47]
Range	The interval between the upper and lower concentration levels of the analyte that have been demonstrated to be determined with acceptable precision, accuracy, and linearity	[38]
LOD and LOQ	The lowest concentration of analyte that can be reliably detected (LOD) and quantified (LOQ) with acceptable precision and accuracy	[45]
Stability	The stability of the analyte in the biological matrix under various conditions, including short-term stability, long-term stability, freeze-thaw stability, and post-preparative stability	[46]

LOD: limit of detection. LOQ: limit of quantitation.

requiring substantial time, effort, and financial investment. Efficient resource management is crucial to balance the costs while maintaining the quality and reliability of bioanalytical methods. Effective strategies can optimize resources, streamline processes, and reduce overall expenses [55]. Automation of sample preparation and data analysis can significantly reduce labor costs, increase throughput, and improve reproducibility. Automated systems can handle large sample volumes with minimal human intervention, reducing the risk of human error and enhancing method consistency. Implementing automated systems for sample preparation, such as liquid handling robots and automated extraction systems, can improve efficiency and consistency [55]. Using software for automated data analysis and reporting can save time and reduce the potential for errors in data interpretation. These systems can quickly process large datasets and generate reports [56,57].

Strategic planning involves prioritizing critical studies and optimizing method development processes to minimize redundant efforts and maximize resource utilization. Effective planning ensures that resources are allocated efficiently, and projects are completed within budget and on time. Focus on studies that are essential for regulatory submissions and critical decision-making in drug development. This helps in directing resources to high-impact projects. Streamline method development processes by adopting systematic approaches such as QbD and DoE. These approaches help in identifying critical method parameters and optimizing them efficiently [58,59]. Collaborating with academic institutions, CROs, and industry partners can provide access to additional resources, expertise, and advanced technologies. Outsourcing certain activities can reduce costs and enhance efficiency. Partnering with academic institutions can provide access to cutting-edge research, specialized equipment, and highly skilled personnel [60]. Outsourcing method development, validation, and sample analysis to CROs can be cost-effective and allow for flexibility in resource allocation. CROs often have specialized expertise and infrastructure to handle complex bioanalytical projects [61]. Collaborating with industry partners can facilitate resource sharing, technology transfer, and joint development of bioanalytical methods. Such partnerships

can leverage the strengths of multiple organizations to achieve common goals. While partnerships with academic institutions and CROs offer access to expertise and resources, they also present challenges. Academic collaborations may face issues such as unclear data ownership, inconsistent quality control, and misaligned timelines. Similarly, CROs may vary in documentation practices and methodological standards, requiring close oversight. Clear agreements and active communication are essential to mitigate these risks and ensure regulatory compliance.

Efficient cost and resource management are essential for the successful development and validation of bioanalytical methods. By implementing automation, strategic planning, and collaborations, bioanalytical laboratories can optimize resources, reduce costs, and maintain high-quality standards. These strategies help balance the financial and operational demands of bioanalytical projects, ultimately contributing to the success of drug development programs.

CONCLUSION

Bioanalytical method development for biologics—including monoclonal antibodies, recombinant proteins, antibody–drug conjugates—and cell and gene therapies presents inherent complexity due to the structural diversity and instability of these modalities. Critical factors such as matrix effects, sensitivity, selectivity, analyte stability, and regulatory compliance must be addressed systematically to ensure reliable data generation. Method validation, tailored to the specific properties of large molecules and gene-based therapies, is essential for clinical and regulatory success. Advanced analytical platforms—such as high-resolution mass spectrometry, ligand-binding assays, digital PCR, and MRM—have significantly improved the sensitivity and selectivity of bioanalytical methods. However, inadequate method development or failure to meet regulatory expectations can result in poor data quality, delayed regulatory approvals, or rejection of pivotal study data. Therefore, a robust, well-validated, and regulatory-compliant bioanalytical strategy is not only scientifically necessary but also critical for de-risking product development. Continued innovation, coupled with strategic planning and compliance, will support the successful characterization and therapeutic monitoring of biologics and gene therapies.

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CELL & GENE THERAPY INSIGHTS

TRANSLATIONAL R&D

Bridging viral immunology and CAR-T therapy: leveraging natural T cell responses to enhance engineered cell therapies

INTERVIEW

"Success ultimately requires thoughtful clinical trial design that evaluates how…immunoengineered products…perform against exhaustion and TME suppression in comparable patient populations."

Ashling Cannon (Editor, BioInsights) speaks to **Catherine Bollard** (Chief Research Officer and Senior Vice President, Children's National Hospital) about translating insights from natural viral antigen-specific T cell responses into improved CAR-T design and clinical performance. They explore how virus-specific T cells demonstrate superior persistence compared to current CAR-T therapies, strategies for preventing T cell exhaustion through TGF-beta resistance, innovative combination approaches merging antigen-specific T cells with CAR-T platforms, and critical regulatory considerations for advancing these therapeutic approaches from bench to clinic.

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Q Can you tell us about your background and how it led to your current work combining viral immunology with cell therapy?

CB I am a pediatric hematologist by training, with dual board certification in pediatrics and hematology pathology. Originally from New Zealand, I moved to the United States in 2000 because I truly believed in the emerging promise of cell therapy. It was an exciting time in the field, and I wanted to be part of that development.

Over 13 years at Baylor College of Medicine, I progressed through the ranks to full professor before Children's National recruited me in 2013 to establish their novel cell therapies program. It was an opportunity to build something from the ground up, which was incredibly appealing.

I am a Professor of Pediatrics and Immunology, Microbiology and Tropical Medicine at The George Washington University and I also hold Faculty affiliations at both Johns Hopkins University and Virginia Tech. I currently serve as the Chief Research Officer and Senior Vice President, as well as Director for the Center for Cancer and Immunology Research at Children's National. This trajectory reflects a career-long commitment to translating cellular immunology discoveries into therapies that can help patients.

Q You have spent decades translating natural viral (antigen)-specific T cell responses into clinical therapies. What translational insights from natural T cell responses to viral or tumor-associated antigens can guide improvements in engineered CAR-T design and clinical performance?

CB The antigen-specific T cell space was the starting point, initially developing T cells targeting Epstein-Barr virus (EBV) antigens for patients who developed EBV-associated lymphomas, after bone marrow transplant, and then for patients who develop lymphoma outside of the context of transplant since 20% to 40% of these lymphomas are EBV positive. Working in the cell therapy and virus-specific T cell spaces revealed that cells employed in gene marking studies persist for decades [1,2]. Unlike gene-engineered receptors, these naturally occurring T cells recognize their cognate antigens through endogenous T cell receptors, and, in the case of EBV, persistent viral antigen provides continuous stimulation for T cell maintenance. Approximately 1% to 2% of B-cells harbor latent EBV in seropositive individuals.

This data revealed the superior persistence capacity of antigen-specific T cells following adoptive transfer especially in the lymphodepleted host. In the transplant setting, these cells demonstrate remarkable efficacy as prophylaxis against viral reactivation or post-transplant lymphoproliferative disorder, achieving over 90% success rates in some studies. Because these cells utilize endogenous T cell receptor recognition rather than engineered constructs, they exhibit a remarkable safety profile. This establishes them as

"[My] trajectory reflects a career-long commitment to translating cellular immunology discoveries into therapies that can help patients."

an optimal T cell platform for additional genetic modifications, such as CAR integration. This potential drove the exploration of combination therapeutic approaches [3,4].

Q T cells targeting viruses demonstrate remarkable memory and persistence that often surpasses current CAR-T therapies. What molecular mechanisms from your viral work are you translating into CAR-T development to enhance long-term efficacy, and what are the key technical hurdles in this translation?

CB The current focus extends beyond viral targeting to explore the targeting of intracellular tumor-associated antigens that conventional CARs cannot address. Traditional CARs are limited to recognizing extracellular antigens, and the number of suitable extracellular targets without significant expression on healthy tissues remains limited. Targeting such shared antigens would result in severe on-target, off-tumor toxicity.

Tumor-associated antigens that parallel viral antigens are predominantly intracellular and processed through the major histocompatibility complex presentation pathway. This creates substantially more targeting opportunities, though conventional CAR approaches are ineffective for intracellular antigens due to their dependence on extracellular recognition domains [5].

Two key translational opportunities have emerged. First, adapting virus-specific T cell manufacturing protocols for tumor-associated antigen-specific T cell production. Second, leveraging tumor-associated antigen-specific T cells as combination platforms with CAR-T therapies. The longevity of virus-specific T cells stems from persistent antigen exposure, which suggests potential strategies such as post-CAR-T vaccination or combination approaches to maintain T cell activation even after tumor clearance.

A critical concern with genetic engineering is the potential for T cell exhaustion. Expanding physiologic T cell populations, whether virus-specific or tumor-associated antigen-specific, may offer advantages over CAR-T cells, which undergo artificial engineering that can lead to overstimulation and premature exhaustion.

The field's diverse approaches to these combination strategies should ultimately provide clarity, though the human model remains the most informative platform. While animal and *in vitro* models provide valuable insights, clinical outcomes in patients remain the definitive measure of therapeutic success.

Q Your viral work has revealed how T cell exhaustion develops in chronic infections. What translational strategies are you applying from this research to prevent CAR-T exhaustion in solid tumors, and how do you envision combining viral immunology principles with CAR-T engineering to overcome the immunosuppressive tumor microenvironment (TME)?

CB T cell exhaustion remains a significant challenge, and checkpoint inhibitor combinations have shown limited success to date. This suggests that other factors in the TME may be driving T cell suppression and exhaustion.

For over 20 years, our team's research has focused on dominant-negative TGF-beta technology. TGF-beta represents one of the most potent tumor-immune evasion strategies employed by human cancers, severely impairing T cell function, particularly the ability to secrete Th1 cytokines and execute cytolytic activity.

Rendering T cells resistant to TGF-beta may prevent CAR-T exhaustion. In our study of EBV-positive Hodgkin lymphoma patients, EBV-specific T cells engineered with the dominant-negative TGF-beta receptor have persisted for at least 5 years, without lymphodepletion, possibly longer [6]. Two patients achieved complete remission and remain disease-free over a decade later, demonstrating the therapeutic potential of this approach.

The question remains: which factors most significantly impact T cell function *in vivo*? Our Cancer Grand Challenge work investigates additional targets, including IL-18 and IL-12, to overcome exhaustion and enhance product potency [7]. Whether these strategies have overlapping mechanisms or differential toxicity profiles requires further investigation, though the dominant-negative TGF-beta receptor has demonstrated notable safety.

Success ultimately requires thoughtful clinical trial design that evaluates how these immunoengineered products, whether resistant to immune suppressive factors or enhanced for activation, perform against exhaustion and TME suppression in comparable patient populations.

Q With your work moving from virus specific T cell therapies to those targeting tumor associated antigens, you are uniquely positioned to develop combination approaches using both antigen-specific T cells and CAR-T. What are the key translational opportunities and challenges in combining these platforms, and how might tumor associated antigen-specific T cells enhance CAR-T efficacy?

CB One key challenge is whether antigen-specific T cells will become exhausted when directly gene-engineered with CAR constructs. While the dominant-negative TGF-beta receptor has not exhausted these cells, as evidenced by their persistence in the study just described, the addition of CAR engineering may alter this dynamic [6].

We are currently evaluating this question both *in vitro* and *in vivo*. Our clinical trial combines three platforms into a single product: the dominant-negative TGF-beta platform, tumor-associated antigen-specific T cells, and CAR-T technology. The approach involves mixing CAR-T cells with tumor-associated antigen-specific T cells engineered with the dominant-negative receptor in a 1:1 ratio before administration.

The therapeutic hypothesis centers on sequential immune activation. CAR-T cells will initially recognize and engage tumor targets through their engineered receptors. Since many solid tumors downregulate MHC expression as an immune evasion mechanism, CAR recognition and subsequent interferon-gamma release should upregulate MHC presentation. This creates the opportunity for TGF-beta-resistant antigen-specific T cells to then recognize and eliminate tumor targets through natural T cell receptor-mediated mechanisms. While we have demonstrated this sequential activation *in vitro*, human TME validation remains crucial for assessing clinical relevance.

This dual-population approach offers unique tracking capabilities since both T cell types carry distinct genetic markers: the CAR construct and the dominant-negative receptor. This will enable precise monitoring of the different T cell populations, including their

persistence and function *in vivo*. If CAR-T cells perform their initial function but subsequently become exhausted, we should be able to detect this transition and measure the longevity of TGF-beta-resistant antigen-specific T cells.

The long-term vision is that persistent antigen-specific T cells engineered to express the dominant negative TGFb receptor will function as biological sinks for TGF-beta released within the TME, thereby maintaining an environment conducive to continued CAR-T function. Hence, this trial should provide valuable insights into both the biological mechanisms and clinical potential of combination cell therapy approaches.

Q Based on your successful translation of multiple antigen-specific T cell therapies and US FDA experience, what are the most critical regulatory and clinical development lessons that apply to CAR-T programs, particularly regarding trial design and manufacturing standards?

CB Manufacturing standardization is critical, and as much of the process as possible should be automated. This is particularly important for autologous cell therapies where patient heterogeneity is inherent. The last thing you want is to introduce additional manufacturing variability on top of that biological variability. Process automation becomes a high priority for ensuring consistency.

While first-in-human dose escalation trials follow established protocols, the secondary endpoints become much more significant for future cell therapy development. These include tracking different T cell populations *in vivo* through sophisticated immunobiologic assays, but there is an often-overlooked component: understanding what is happening on the tumor side.

To that end, as part of our Cancer Grand Challenges team NexTGen funded by the NCI, CRUK and The Mark Foundation for Cancer Research, we work closely with patient advocates to educate families about the importance of post-treatment biopsies. These samples are crucial for determining why therapies succeed versus why they fail, and more importantly, understanding the mechanisms of resistance. Moreover, the cell therapy community as a whole could make substantial collective progress through harmonized correlative studies. Learning from both treatment failures and successes across programs would accelerate the field significantly.

Previous experience has demonstrated that antigen loss represents a potent immune escape mechanism. Therefore, targeting multiple antigens within a single product is a priority for many groups developing cell therapies for solid tumors. While the optimal number of targets remains unclear, it is likely to be more than one. Such a multi-antigen strategy would address one of the most predictable resistance mechanisms encountered in cell therapy.

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BIOGRAPHY

Catherine Bollard received her medical degree at the University of Otago in Dunedin, New Zealand. She is board certified both in pediatrics and hematology. She worked in New Zealand and London, before moving to Houston, Texas, in 2000 where she was a Professor of Pediatrics, Medicine, and Immunology at Baylor College of Medicine (BCM). In August 2013, she was recruited to Washington, DC, to join Children's National Hospital and George Washington University. She is currently the Senior Vice President & Chief Research Officer and the Dr Robert J and Florence T Bosworth Distinguished Professor of Cancer and Transplantation Biology Research and the Director of the Center for Cancer and Immunology Research. Catherine is a Professor of Pediatrics and of Microbiology, Immunology, and Tropical Medicine at George Washington University and the Associate Center Director for Translational Research and Innovation at the GW Cancer Center. She is a member of the American Society for Clinical Investigation and the Association of American Physicians (AAP), a past president of the International Society for Cellular Therapy (ISCT), and the current President of the Foundation for the Accreditation of Cellular Therapy (FACT). Catherine was a member of the Cellular, Tissues, and Gene Therapies Advisory Committee of the FDA from 2015 to 2019 and was an associate editor for the journal *Blood*, 2014–2021. She is currently the Editor-in-Chief of *Blood Advances*. Catherine has over 250 peer reviewed publications and has been independently NIH-funded for more than 15 years. Her bench and translational research focuses on improving outcomes for patients after transplant and on the development of novel cell therapies for cancer and virus-associated diseases.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

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CELL & GENE THERAPY INSIGHTS

TRANSLATIONAL R&D

REVIEW

Autologous CAR-T cell immunotherapy for autoimmune diseases: a systematic review

Tisha Singhal and John Maher

Autologous CAR-T cell immunotherapy has emerged as a promising treatment for autoimmune disease, achieving high remission rates with manageable side effects. However, concerns remain regarding long-term safety, durability, and manufacturing scalability. Aim(s)/objectives: this systematic review assesses the therapeutic potential of autologous CAR-T cells. The feasibility of autologous CAR-T manufacturing and future directions will also be discussed. Methods: 27 studies (2019–2025) from 3 databases were reviewed to assess the clinical efficacy and safety of autologous CAR-T cells in autoimmune disease. Of 131 patients treated, 58% achieved complete remission, while an additional 35.8% demonstrated clinically significant improvement. Mild cytokine release syndrome (grades 1–2) was reported in 51.9% and 4.6% experienced immune effector cell-associated neurotoxicity syndrome. Other side effects, often linked to lymphodepleting chemotherapy, were manageable. Limitations of the review include a small sample size, a non-randomized design, and a short follow-up. Conclusions: CAR-T immunotherapy achieves effective remission of several autoimmune disorders with manageable toxicity. Larger, longer-term controlled trials are needed to confirm these findings and the durability of response. Emerging innovations like self-regulating CAR-T cells and manufacturing developments such as T-Charge™ and *in vivo* manufacturing may improve efficiency and scalability for widespread clinical use.

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INTRODUCTION

Chimeric antigen receptor T-cell (CAR-T) immunotherapy is an emerging therapeutic modality that has achieved significant success in the treatment of refractory or relapsed B-cell malignancies [1]. There are currently seven Food and Drug Administration (FDA)-approved CAR-T cell therapies available for the treatment of cancer. More recently, CAR-T cell immunotherapy has shown striking early promise in the treatment of autoimmune diseases with the potential for future FDA approvals

in this field [2]. In this systematic review, we summarize the rapidly evolving field of CAR-T cell immunotherapy for refractory autoimmune disease.

Structure of chimeric antigen receptors

Chimeric antigen receptors are recombinant fusion proteins that consist of (from inside out) an intracellular signaling domain (containing one or more elements), a transmembrane domain, a hinge region and an antigen binding domain [3]. CAR-T cells are generated by the genetic modification of T cells to either transiently or stably express these synthetic receptors. The key defining property of a CAR is that it confers on polyclonal T cells the ability to recognize a designated cell surface target (or targets) in an MHC-independent manner, contrasting with the MHC-restricted nature of antigen recognition by most T-cell receptors (TCR). Although many architectures have been described, five generations are well known in the field, and these are schematically illustrated in Figure 1.

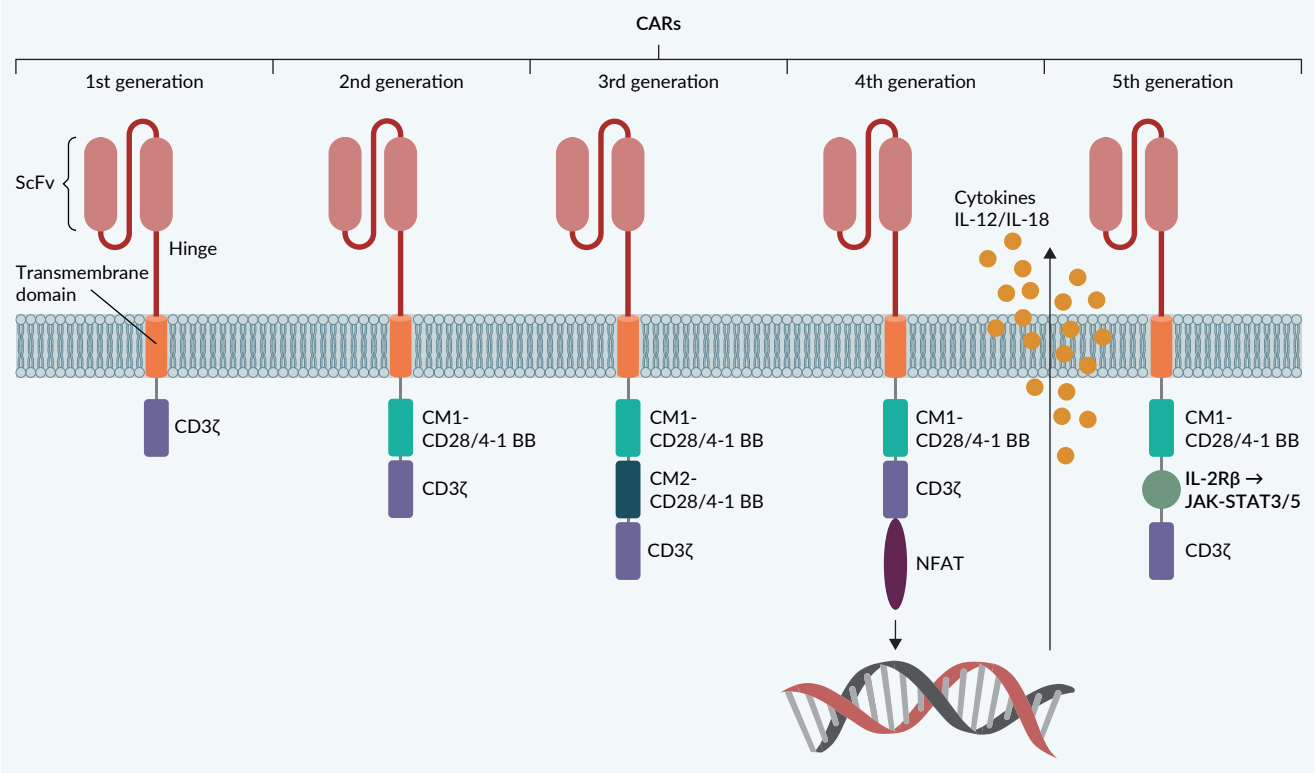
First-generation CAR-T cells consist of an extracellular antigen-binding domain and an intracellular activation domain, most commonly derived from CD3ζ [4]. However, pre-clinical models have demonstrated that first-generation CARs fail to elicit robust cytokine production and T cell proliferation upon activation, leading to therapeutic failure upon clinical evaluation [5]. To address this, second-generation CAR-T cells incorporate an additional costimulatory domain, most commonly derived from either CD28 or 4-1BB [6]. The presence of a costimulatory domain improves T cell proliferation, cytokine release, and survival upon CAR cross-linking [7]. Third-generation CARs incorporate two or more costimulatory domains, although these have not achieved further major clinical impact when compared to second-generation designs. Fourth-generation receptors include an armoring strategy to enable the CAR-T cells to produce cytokines such as interleukin (IL)-12 or IL-18. Often cytokine release is restricted to the period immediately after CAR-T cell activation, for example, through the incorporation of a nuclear factor of activated T cells (NFAT) mini-promoter, which controls expression of the armoring cytokine. Finally, fifth-generation CARs incorporate JAK kinase binding elements within the CAR endodomain to further enhance activation-dependent T-cell proliferation [8]. All FDA-approved CAR-T cell treatments are of second-generation design, containing either CD28 or 4-1BB [4].

Manufacture and administration of autologous CAR-T cells

Clinical experience of CAR-T cell immunotherapy largely derives from the treatment of B-cell malignancy and multiple myeloma. In that setting, autologous CAR-T cells are engineered using the patient's own T cells, as outlined in Figure 2 [9]. The first step involves peripheral blood mononuclear cell isolation, which is generally performed using leukapheresis or, alternatively, using a blood draw from the patient [10]. In some cases, $CD4^+$ and $CD8^+$ T cells may be immunomagnetically purified from the starting material using microbeads or through depletion of non-T cells [9]. The next step is T cell activation, which is generally undertaken using magnetic beads, nanoparticles or plasticware pre-coated with anti-CD3 and anti-CD28 antibodies [11]. The CAR transgene is next introduced into the T cells, most commonly using a lentiviral or retroviral vector [12]. Alternatively, mRNA transfection may be used to achieve transient CAR expression. Transduced/transfected T cells are then expanded to achieve the required dose, which takes approximately 1–2 weeks. Various cytokines such as IL-2, IL-7, and/or IL-15 are used to provide optimum conditions for the growth of the T cells [9]. Finally, CAR-T

FIGURE 1

Structural attributes of five CAR generations.



CAR: chimeric antigen receptor. CD: cluster of differentiation. CM: co-stimulatory module. Gen: generation. JAK-STAT: janus kinase-signal transducer and activator of transcription. IL: interleukin. NFAT: nuclear factor of activated T-cells. scFv: single chain antibody fragment.

cells are concentrated, cryopreserved, and subjected to quality control release assays to confirm purity, potency, and sterility of the drug product. Prior to infusion of the cells, patients are generally conditioned using lymphodepleting chemotherapy. Lymphodepletion is of key importance since it depletes endogenous lymphocytes, leading to reduced competition for homeostatic cytokines and more extensive *in vivo* expansion of CAR-T cells [13]. Within days of completion of this step, CAR-T cells are thawed and infused into the patient [11].

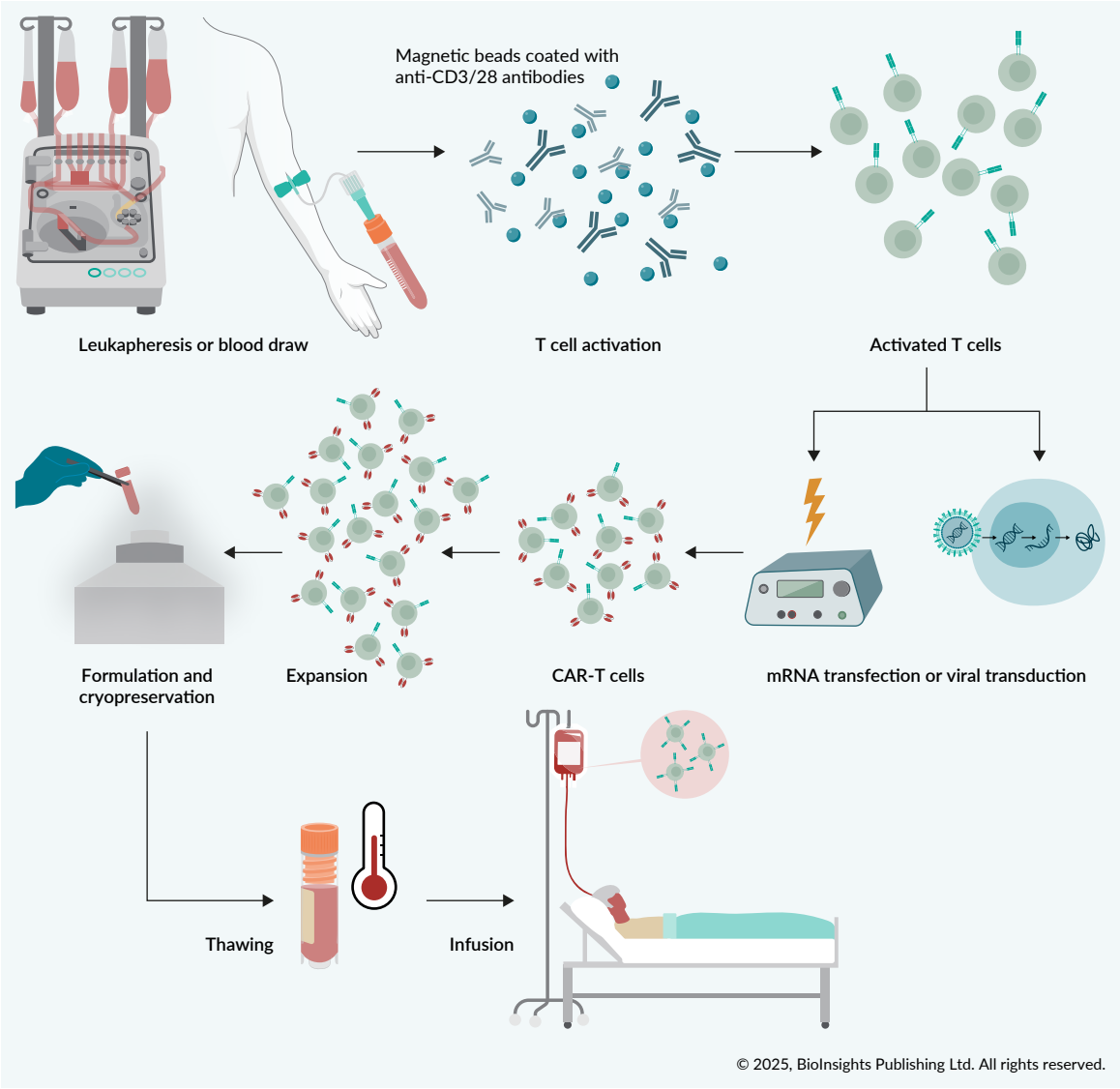
B-cell involvement in autoimmune disease

All approved CAR-T cell products are targeted against the B-cell lineage antigens. However, B cells are also known to play a role in driving autoimmune diseases [14]. Hence, many therapies that target B cells have been employed for the treatment of autoimmune disease, most notably rituximab, which is a chimeric anti-CD20 monoclonal antibody [15].

There are two key mechanisms by which B cells contribute to the pathogenesis of autoimmune disease, namely autoantibody production and antigen-presenting cell (APC) function [16]. Autoantibodies can form immune complexes by binding to self-antigens, which cause tissue damage via complement activation and antibody-dependent cell-mediated cytotoxicity [17,18]. B cells can also present self-antigens via MHC class II molecules to $CD4^+$ T cells. The latter then undergo activation and differentiate to become T follicular helper (TFH) cells. B cells also produce both pro-inflammatory

FIGURE 2

Outline of the *ex vivo* manufacturing processes used to engineer autologous CAR-T cells.



and anti-inflammatory cytokines [14]. Pro-inflammatory cytokines promote B cell proliferation, TFH cell differentiation, and germinal center formation, driving autoimmunity in diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS) [19]. By contrast, regulatory B cells produce IL-10, transforming growth factor β, and IL-35, all of which are anti-inflammatory cytokines that suppress immune responses [20,14]. Accordingly, it has been hypothesized that an imbalance between pro- and anti-inflammatory cytokine production can contribute to the development and progression of autoimmune diseases [14].

Potential for CAR-T cell immunotherapy of autoimmune disease

As indicated above, CAR-T cells target one or more specific antigens in an MHC-independent manner. All currently

approved CAR-T cell products for cancer engage one of two antigens: CD19 or B cell maturation antigen (BCMA) [21]. CD19 is expressed on B cells from a very early stage, and its expression is maintained throughout multiple stages of B cell differentiation and activation [22]. Logically, given the role of B cells in autoimmunity, there has been recent interest in the application of these approved and emerging CAR-T cell products for the treatment of autoimmunity. The broad expression and B-cell specificity of CD19 make it a key target for CAR-T cells to decrease autoantibody production and reset immune balance [23]. Similarly, BCMA has an overlapping but distinct profile of expression since it is also found on plasma cells and late-stage B cells. Conceptually, this means that BCMA-CAR-T cells can specifically deplete antibody-secreting cells without affecting earlier B cell populations, making it an attractive target for experimental CAR-T cell treatment of autoimmune disease [24].

METHODS

This systematic review adheres to the PRISMA guidelines 2020 [25]. Studies were sourced from three databases: PubMed, ScienceDirect, and BMJ Journals. The search was carried out using the keywords ("Autoimmune") AND ("CAR-T cell" OR Chimeric antigen receptor T-cell) AND ("autologous") in all 3 databases.

The eligibility criteria were formed based on the Population, Intervention, Comparison, Outcome (PICO) network [26]. Studies were selected based on the following inclusion criteria:

- Full article available in English language
- Preclinical and clinical trials including *in vivo* animal models and *in vitro* cell line models
- Autologous CAR-T cells intervention
- Exposure is an autoimmune disease
- Reported outcomes of safety and effectiveness of therapies (quantitative and/or qualitative)

Secondary research articles, including systematic reviews, literature reviews, editorials, and letters, articles with no published results (neither qualitative nor quantitative), and *in vivo* expanded CAR-T cells were excluded.

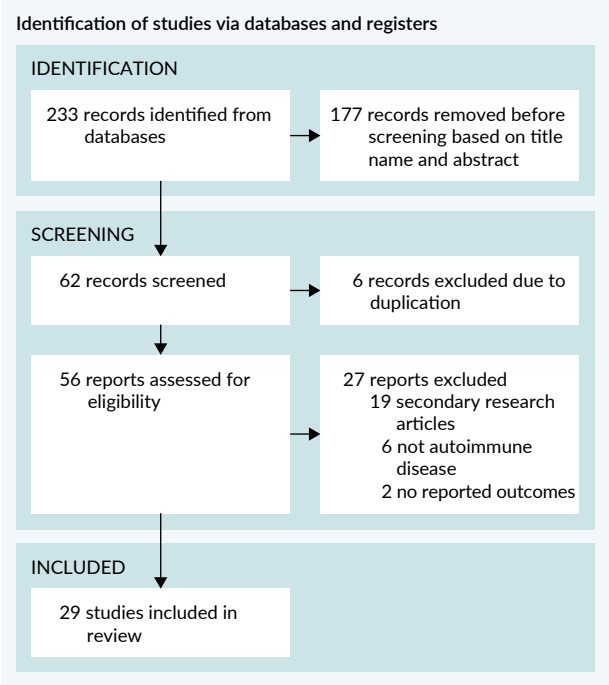
There were no restrictions based on the date or geographical location of the study. Additionally, participant characteristics, such as age, sex, gender, and minimum follow-up duration, were not limited. This inclusive approach was designed to gather as much data as possible. Peer review was not an exclusion (gray literature, such as abstracts and conference pieces, was included).

RESULTS

The selection process that yielded the studies included in this systematic review is summarized in Figure 3. Twenty-nine studies were identified, all of which were published between 2019–2025 (Table 1) [27–55]. Twenty-five were clinical trials (86.2%) and four were pre-clinical studies (13.8%). Two of the pre-clinical trials were *in vivo* mouse models and two were *in vitro* models (one using established cell lines and one using patient-derived primary cells from 9 patients). In 25 studies (86.2%), CD19 was the target antigen, while in the remainder, targets consisted of BCMA alone, BCMA and CD19, or CD19 and CD20. In total, 83 subjects were included in all the clinical studies, of whom 44 (53%) were reported to have achieved remission within a year following CAR-T cell immunotherapy. Among the 39 subjects for whom remission was not explicitly reported, 6 achieved near-complete remission, while 31 demonstrated major symptomatic and

FIGURE 3

Studies of CAR-T cell immunotherapy of autoimmunity identified following PRISMA guidelines 2020.



The flowchart shows the process of identifying and selecting studies. A total of 227 studies were identified across 3 databases: PubMed, ScienceDirect, and BMJ Journals. Based on title and abstract, 167 studies were removed, leaving 60 studies to be screened for duplications. Six duplicated articles were removed. Full-text analysis was conducted on the remaining 54 studies using the eligibility criteria. As a result, 27 studies were included in the final review.

clinical improvement (Table 2). Study 21 focused on evaluating deep B cell depletion in lymphoid organs rather than assessing disease remission or symptom changes. Fifty-one subjects (60%) were confirmed to have cytokine release syndrome (CRS), which is a well-known adverse reaction attributable to CAR-T cell immunotherapy. Most CRS cases were mild or self-limiting (i.e., grade 1 or grade 2), apart from a single grade 3 episode. All CRS episodes resolved with supportive care alone or together with tocilizumab, a humanized IL-6 receptor blocking antibody. Immune effector cell-associated neurotoxicity syndrome (ICANS) was observed in 5 patients (4 grade 1 and 1 grade 4). One patient developed pneumonia and another developed influenza, both of which resulted in hospitalization. Other mild adverse events reported were hematological toxicities (leukopenia, neutropenia, thrombocytopenia—all likely due to lymphodepleting chemotherapy), headache, nausea, vomiting, and fever. One treatment-related death was reported in study 28. While death was associated with excessive CAR-T expansion and secondary hemophagocytic lymphohistiocytosis (HLH), several pre-existing risk factors, including advanced age, severe organ dysfunction, prolonged cytopenias, and the presence of a *TET2* mutation, also likely played a significant role.

All clinical studies were non-randomized and unblinded—essentially early 'proof-of-concept' studies rather than rigorous clinical trials. Hence, the risk of bias is considered high, and the certainty of evidence is low.

DISCUSSION

Data summarized in this systematic review demonstrate proof of concept that autologous CAR-T cells are highly effective in the treatment of a wide range of refractory autoimmune diseases, with mild side effects observed. However, there are several limitations to the evidence provided by these studies. Firstly, all studies have small sample sizes. Across 29 clinical studies, there were only 83 participants, which is unlikely to be truly representative of the broader population, limiting the external validity of the results. Furthermore, the results are more likely to be affected by anomalies, and this variability reduces the reliability of the findings. Thirdly, the lack of control groups in all these trials makes it difficult to evaluate the true effectiveness of CAR-T cell therapy. It is possible that the placebo effect or other confounding factors, such as lifestyle changes, may have contributed to patient improvement. Lastly, the studies are limited by a relatively short duration

TABLE 1

Pre-clinical and clinical studies of autologous CAR-T cell therapy for the treatment of autoimmune diseases.

Study	Disease	CAR-T target	No of subjects	Outcome/result	Reference/ partner	Year
1	SLE—murine lupus	CD19	Pre-clinical	Depletion of $CD19^+$ B cells sustained for 6 months; survival significantly improved with many mice alive beyond 15 weeks, which is the typical lifespan of the strain; kidney histopathology showed reduced glomerular infiltrates and IgG deposits	[27]	2019
2	APLS and DLBCL	CD19	1	Normalized anticardiolipin antibody levels from 97.3 MPL to <9.4 MPL; no thromboembolic events after therapy; CD19 B cell aplasia sustained; DLBCL remained in remission, and anticardiolipin levels remained normal at 1-year follow-up	[28]	2020
3	SLE—murine lupus	CD19	Pre-clinical	Anti-CD19 CAR-T with TBI pre-conditioning eradicated almost all circulating $CD19^+$ B cells by one week after transfer; depletion was more sustained than had been achieved using CD19-specific antibody depletion; 22% of CAR-T-treated mice had skin lesions compared to 80% of PBS-treated control mice at 22 weeks; no significant differences in autoantibodies or proteinuria	[29]	2021
4	SLE	CD19	1	Complete B-cell depletion, with CAR-T cell levels peaking at 27.69% of total circulating T cells by day 9; rapid decrease in dsDNA autoantibodies from >5000 U/mL to 4 U/mL; proteinuria decreased from 2000 mg/g to <250 mg/g; SLEDAI score decreased from 16 to 0.5; no adverse events (e.g., CRS, ICANS)	[30]*	2021
5	SLE	CD19	5	All 5 patients achieved SLE remission (DORIS criteria) at 3 months; SLEDAI-2K score at 3 months: 0 in 4/5 patients (one had a score of 2, possibly due to previous glomerular damage); normalization of anti-dsDNA antibodies and complement levels; proteinuria decreased, and nephritis ceased in all patients; drug-free remission was maintained up to 17 months; mild CRS, no ICANS	[31]	2022
6	MG	BCMA (RNA encoded CAR)	14	Significant improvements at week 12: MG-ADL: −5.9 [−9, −2.8]†, QMG: −7 [−11, −3], MGCS: −14 [−19, −9], MG-QoL-15r: −9 [−15, −3]; 89% of participants had improvements in MG scales at week 12; 3 of 7 participants in Arm-2 achieved minimal symptom expression (MG-ADL ≤1); no need for IVIG in 2 patients after treatment; no dose-limiting toxicity, CRS, or neurotoxicity; common AEs: headache, nausea, vomiting, fever (resolved within 24 hours)	[32]; Cartesian Therapeutics	2023
7	SLE	CD19	6	Decreased levels of IL-6, IL-10, and TNF-α; increased levels of IL-7 and BAFF 3 months post-infusion; reduction in SLE-associated autoantibodies (anti-dsDNA, anti-ssDNA, anti-histone, anti-SSA/Ro52, and others) in 5 out of 6 patients; minimal to mild effects on pre-existing immunity to infectious agents and vaccines; all six achieved and remained in drug-free remission at 3 months	[33]; Cabaletta Bio	2023
8	Myositis and ILD associated with ASS	CD19	1	Rapid clinical improvement, reduced muscle pain/weakness; 8 months after treatment: improved Physician Global Assessment scores, muscle strength, and pulmonary function; also, no detectable myositis signs on MRI scan; normalization of serum muscle enzymes (AST, ALT, CK, LDH) and inflammatory cytokines (IL-1, IL-6, IL-13, IFN-γ); B-cell depletion and subsequent recovery in B-cell counts and Igs; anti-Jo-1 antibody levels decreased	[34]	2023
9	NMOSD	BCMA	12	Eleven out of 12 treated patients achieved drug-free remission with no relapses; grade 3 or higher cytopenias in all patients; 7 patients (58%) developed infections; no grade 4 infections; CRS observed in all patients (grade 1–2); no grade 3 CRS or new-onset neurotoxicity; 83% of patients (5/6) showed negative AQP4-IgG levels by 6 months; significant improvement in EDSS, ambulation, visual acuity, and bowel/bladder function; decreased Igs levels in all patients; 25% of patients showed anti-drug antibodies post-infusion	[35]	2023
10	MG	CD19	1	A 33-year-old woman with severe, refractory, anti-AchR-positive generalized MG (MGFA class V) was treated with anti-CD19 CAR-T cells, which remained detectable at day 62 (0.5 cells/μL, 0.19% of total CD3 T cells); anti-AchR autoantibody levels decreased from 2434 nmol/mL at baseline to 718 nmol/mL at day 62 (~70% reduction), while protective vaccination IgG titers were maintained; adverse events included self-limiting grade 1 transaminitis; clinical improvement observed over the first 2 months was as follows: increased arm-holding duration; enhanced walking ability without supportive devices; reduction in Besinger disease activity and QMG scores; minimal immunosuppression was maintained, with plans for withdrawal; at the last reported follow-up (day 62), substantial functional improvement and marked reduction in pathogenic autoantibodies were reported, with no B-cell reconstitution	[36]	2023
11	ASS	CD19	1	44-year-old woman with severe refractory ASS. CAR-T cells expanded >1000-fold (peak 1524.2/μL on day 10) with complete B-cell depletion for 58 days; mild CRS (grade 1) occurred and resolved with tocilizumab; transient grade 1 ICANS (dizziness) occurred on day 7, which resolved after a short dexamethasone course; no higher-grade toxicities reported CK decreased from 4298 U/L at baseline to 99 U/L by day 150; myoglobin decreased from 2945 μg/L to 53 μg/L; ALT decreased from 317 U/L to 37 U/L; MRI at 3 months showed complete resolution of muscle inflammation; manual muscle testing improved from 103/150 at baseline to 150/150 by day 150; endurance improved as follows: Sit-to-Stand repetitions from 0 to 13; walking distance from 50 m to 2000 m; major clinical response was confirmed by ACR/EULAR TIS (82.5 at day 90; 97.5 at day 150); at the latest follow-up (day +150), the patient was in drug (including glucocorticoid)-free remission, with complete resolution of myositis, arthritis, and lung involvement	[37]	2024

*These references contain overlapping patients which have been accounted for and excluded from overall totals. †Values are presented as mean change from baseline with 95% confidence intervals in square brackets. Negative changes indicate improvement in disease severity scores. ACPA: anti-citrullinated peptide antibody. ACR/EULAR TIS: American College of Rheumatology/European League Against Rheumatism Total Improvement Score. AE: adverse effect. ALT: alanine aminotransferase. ANA: anti-nuclear antibody. anti-AchR: acetylcholine receptor. anti-GAD65: glutamic acid decarboxylase 65kDa. anti-VGCC: anti-voltage gated sodium channels. APLS: antiphospholipid syndrome. AQP4: aquaporin 4. ASS: anti-synthetase syndrome. AST: aspartate transaminase. BAFF: B cell activating factor. BCMA: B cell maturation antigen. CABA-201: Cabaletta 201. CAT-BM: Cutaneous assessment tool—binary method. cCAR: compound CAR. CK: creatine kinase. CMAS: Childhood Myositis Assessment Scale. CMV: cytomegalovirus. CRP: C-reactive protein. CXCL9: CXC motif chemokine ligand 9. DAS-28-CRP: Disease Activity Score using 28 joint counts and C-reactive protein. DLBCL: diffuse large B cell lymphoma. DLCO: diffusing capacity of lungs for carbon monoxide. DORIS: definition of remission in systemic lupus erythematosus. dsDNA: double stranded DNA antibodies. EDSS: Expanded Disability Status Scale. EUSTAR: European Scleroderma Trials and Research Group. EUSTAR-AI: European Scleroderma Trials and Research Group Activity Index. FDC: follicular dendritic cell. FVC: forced vital capacity. GABAergic: gamma-aminobutyric acid. GM-CSF: granulocyte-macrophage colony-stimulating factor. HAQ-DI: Health Assessment Questionnaire Disability Index. HLH: hemophagocytic lymphohistiocytosis. HSV-1: herpes simplex virus 1. IFN-γ: interferon gamma. Igs: immunoglobulins. IIM: idiopathic inflammatory myositis. IL: interleukin. ILD: interstitial lung disease. IMNM: immune-mediated necrotising myopathy. IVIG: intravenous immunoglobulin. LEMS: Lambert-Eaton myasthenic syndrome. LDH: lactate dehydrogenase. LLDAS: Lupus Low Disease Activity State. LN: lupus nephritis. mcPV: mucocutaneous pemphigus vulgaris. MG: myasthenia gravis. MGCS: Myasthenia Gravis Composite Score. MG-ADL: Myasthenia Gravis Activities of Daily Living. MGFA: Myasthenia Gravis Foundation of America. MMT8: Muscle Memory Test 8. MPL: IgM phospholipid units. MRI: magnetic resonance imaging. mRSS: Modified Rodnan Skin Score. MS: multiple sclerosis. NMOSD: neuromyelitis optica spectrum disorder. OCB: oligoclonal bands. PBS: phosphate buffered saline. PGA: patient global assessment. QMG: Quantitative Myasthenia Gravis Score. QoL-15r: Myasthenia Gravis Quality of Life 15-item Scale—Revised. RA: rheumatoid arthritis. RF: rheumatoid factor. RNA: ribonucleic acid. RTX: rituximab. SLE: systemic lupus erythematosus. SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000. SSc: systemic sclerosis. TBI: total body irradiation. TET2: ten-eleven-translocation 2. TNF-α: tumor necrosis factor alpha.

TABLE 1 (CONT.)

Pre-clinical and clinical studies of autologous CAR-T cell therapy for the treatment of autoimmune diseases.

Study	Disease	CAR-T target	No of subjects	Outcome/result	Reference/ partner	Year
12	Severe treatment-refractory stiff-person syndrome	CD19	1	Reduced stiffness and pain, walking speed increased >100% (0.37–0.83 m/s), and walking distance improved to >6 km; GABAergic medication was reduced by 40%; fatigue decreased from 48 to 40 on the Fatigue Severity Scale; anti-GAD65 titers decreased (1:3,200 to 1:320); low-grade CRS (fever, hypotension) treated with paracetamol, dexamethasone, and tocilizumab; mild liver transaminase elevation, resolved by day +45	[38]; Kyverna Therapeutics	2024
13	Progressive MS	CD19	2	Patient 1: walking distance improved from 400–700 m (EDSS 4.0); no new neurological symptoms; B cells depleted by day 2; no reconstitution by day 100; reduced OCBs and IgG levels in CSF by day 14 and sustained through day 64; CRS grade 1 with no ICANS; transient mild liver enzyme increases Patient 2: EDSS remained stable throughout. No new neurological symptoms; no change in OCBs or IgG levels. No CRS or ICANS; transient mild liver enzyme increases	[39]; Kyverna Therapeutics	2024
14	B-cell lymphoma in a patient with SLE and APLS	CD19	1	Negative lupus anticoagulant, anticardiolipin (IgG and IgM), and anti-β2 glycoprotein I antibodies by Day 79 post-infusion; 1 year later, the patient achieved sustained complete remission of all 3 aPLS antibodies (lupus anticoagulant, anticardiolipin, anti-β2 glycoprotein I); remission was maintained without thromboembolic events; CD19+ B cells were profoundly depleted, and ANA titers also became negative; despite ongoing anticoagulation, no recurrence of thrombotic events was observed	[40]	2025
15	SLE	CD19	2 (pediatric)	Patient 1: complete resolution of facial rash, ulcers, and proteinuria by day 60; complement C3 normal by day 28; anti-dsDNA negative by month 4; SLEDAI-2 K score decreased from 12 to 0; Grade 1 CRS and grade 1 ICANS; discontinued glucocorticoids and immunosuppressants. Patient 2: resolution of pleurisy and hematuria; anti-dsDNA normalized; proteinuria reduced from 28 to 13 mg/kg/day; SLEDAI-2 K score decreased from 12 to 4; kidney biopsy showed improved lupus nephritis; grade 1 CRS and grade 1 ICANS; discontinued glucocorticoids and immunosuppressants	[41]; Chongqing Precision Biotech Co. Ltd	2024
16	Concomitant MG and LEMS	CD19	2	Patient 1: major neurological improvements, including complete resolution of the Trendelenburg sign by day 60, gait recovery, and independence from wheelchair; achieved 8 km walking and 26 km e-biking; pulmonary vital capacity improved to 3.9 L; strength of the patient's lid and small ocular muscles continued to show major improvements; stable at 4-6 months post-infusion, and anti-AChR and anti-VGCC autoantibodies normalized. Patient 2: achieved 3 km walking at day 60, improved pulmonary function to 3.3 L, regained independence from wheelchair by day 4; stable at 4-6 months post-infusion, and anti-AChR and anti-VGCC autoantibodies normalized	[42]; Kyverna Therapeutics	2024
17	SLE, IIM, SSc	CD19	SLE (8), SSc (4), and IIM (3)	SLE: all 8 achieved DORIS remission, resolved proteinuria, C3 normalized, anti-dsDNA absent; IIM: all 3 had ACR-EULAR major clinical response; SSc: all 4 had decreased EUSTAR score (-4.2); 10 patients had CRS (grade 1); 1 IIM patient had CRS (grade 2); 1 IIM patient with mild ICANS; 1 SLE patient with pneumonia resulting in hospitalization, which resolved; all patients discontinued immunosuppressive therapy; at a median of 15 months of follow-up (range 4–29), all remained in remission without relapse	[43]	2024
18	SLE, MS, mcPV, RA, SSc, IIM	CD19	Pre-clinical	CABA-201 demonstrated >90% cytotoxicity against autologous CD19+ B cells across all diseases, with antigen-specific activation confirmed by upregulation of CD69 and CD25; cytotoxic activity was sustained over four rounds of serial B cell exposure, and cytokine secretion (IFNγ, TNFα, IL-2, GM-CSF) was significant but within a log10 range of control CD19-specific CAR-T cells; no significant differences in cytotoxicity were observed between healthy donors and autoimmune disease donors; no cytotoxicity/cytokine production observed when CABA-201 was co-cultured with healthy human primary bladder epithelial cells or small intestinal epithelial cells, indicating no cross-reactivity against these tissues	[44]; Cabaletta Bio	2024
19	Juvenile dermato-myositis	CD19	1 (pediatric)	A 12-year-old boy received CD19 CAR-T cells (1×106 cells/kg), which expanded with a peak of 32.7/μL on day 7 and became undetectable by day 28; toxicities included grade 1 CRS (fever), grade 2 anaemia, and grade 4 neutropenia; no infections observed; clinical response began at week 4; by week 34, PGA improved from 10/10 to 1/10, CMAS from 36/52 to 50/52, and CAT-BM from 9/17 to 2/17; muscle strength normalised; MRI showed resolution of myositis; skin ulcerations and calcinosis markedly improved (residual Gottron signs and calcinosis still resolving); type I IFN score normalized by week 24, with CXCL9 and CXCL10 decreasing into the normal range; at last follow-up (8 months post-infusion), the patient remained off all immunosuppressive therapy, with sustained improvement of muscle and skin disease and ongoing resolution of calcinosis	[45]	2024
20	RA and DLBCL	CD20-CD19	1	Zamtocabtagene autoleucel induced sustained drug-free remission of RA and partial response/stable disease for DLBCL; RA symptoms significantly improved post-CAR-T, with a decrease in RF levels from 1200 IU/mL to 13 IU/mL, and low ACPA levels, indicating immunological remission; DLBCL showed a partial response initially, but by week 48, a complete metabolic response was confirmed; no significant ICANS, infection, or tumor lysis syndrome occurred. Grade I CRS	[46]; Miltenyi Biotec	2024

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TABLE 1 (CONT.)

Pre-clinical and clinical studies of autologous CAR-T cell therapy for the treatment of autoimmune diseases.

Study	Disease	CAR-T target	No of subjects	Outcome/result	Reference/ partner	Year
21	SLE, SSc	CD19	SLE (6), SSc (2)	CD19+ and CD20+ B cells were completely depleted in lymph nodes, while plasma cells, T cells, and macrophages remained unchanged; follicular structures were disrupted, and FDCs were depleted in the lymph nodes; plasma cells showed reduced proliferation rates after CAR-T cell therapy compared to rituximab treatment; non-lymphoid organs (colon, kidney, gallbladder) also showed complete B-cell depletion, with T cells and macrophages present; no significant difference in the depletion of circulating B cells or immunoglobulin levels between CAR-T and RTX-treated patients	[47]	2025
22	SLE with LN	BCMA-CD19 cCAR	13	Patients 1 and 2 had both DLBCL and SLE; both achieved symptom and medication-free remission from SLE and complete remission from lymphoma; 3 months post-treatment, P3–P13 (except P11) were negative for all autoantibodies, including those from long-lived plasma cells; complement levels normalized; they also achieved symptom and medication-free remission at 46-month follow-up; 10 LN patients showed significant renal function improvement ≤90 days post-cCAR; B cell recovery occurred 2–6 months post CAR-T; SLEDAI reduced from 10.6 (baseline) to 2.7 (3 months); Therapy was well-tolerated with mild CRS	[48]; iCell Gene Therapeutics; CAR Bio Therapeutics	2024
23	IMNM	CD19	1	33-year-old male with refractory IMNM received CABA-201, a fully human 4-1BB + CD3ζ CD19 specific CAR-T therapy; the infusion was well tolerated with no CRS, ICANS, or serious adverse events reported during 4 months of follow-up; CK levels decreased, and muscle strength improved (MMT8 score); peripheral B cells were rapidly depleted and undetectable by day 15, with repopulation beginning at 8 weeks and consisting predominantly of transitional naïve B cells; autoantibodies to SRP-9, SRP-54, SRP-72, and Ro-52 declined by 74%, 54%, 81%, and 70%. respectively, while vaccine- and pathogen-associated antibodies remained stable; at 16 weeks post-infusion, the patient remained off all other immunosuppressive therapy	[49]	2024
24	LEMS	CD19	1	LEMS symptoms improved: QMG score reduced from 18 (baseline) to 9 (day 85), Besinger score from 1.5 to 0.5, and MG-ADL score from 13 to 4; walking distance increased from 11–90 m, and leg/arm holding times improved; patient was able to mobilize independently, e.g., changing position in bed from day 29, shaving from day 41, which hadn't been possible for the preceding 2 years; VGCC Abs reduced to 40% by day 43; no GABA B receptor Abs detected at baseline; grade 1 nausea due to lymphodepleting therapy managed with antiemetics; fever and hypotension due to CRS after day 2 of CAR-T infusion resolved by day 7; non-infectious diarrhea day 5 (due to lymphodepletion); no observed infections	[50]; Kyverna Therapeutics	2024
25	SLE, IIM, SSc	CD19	Pre-clinical; SLE (3), IIM (3), SSc (3)	The fully human Hu19-CD828Z CAR was expressed in T-cells from SLE, SSc, and IIM patients, showing comparable transduction efficiency to healthy donors (52–69% CAR+). CAR-T cells exhibited robust CD19-dependent proliferation and dose-dependent cytotoxicity against autologous B cells and CD19+ NALM-6 cells, with minimal activity against CD19-negative targets, confirming CD19 specificity; cytokine release (IFN-γ, TNF-α, IL-2, IL-6, and IL-1β) increased significantly in co-cultures with CD19+ targets and correlated with CD19 expression levels; patient-derived CAR-T cells produced lower levels of inflammatory cytokines (IFN-γ, TNF-α, IL-6) compared to healthy donor CAR-T cells	[51]; Kyverna Therapeutics	2025
26	RA	CD19	1	Patient had persistently active RA with a DAS-28-CRP score of 7.46 and a CRP level of 104 mg/L (normal <5 mg/L) prior to CAR-T therapy. The patient received an infusion of 1×106 CAR-T cells/kg; at 100 days post-therapy, the patient was in drug-free remission with a DAS-28-CRP score of 2.5, no neurological sequelae, and normalized inflammatory markers; RF and ACPA levels decreased by more than 80%; post-infusion adverse events included grade 3 CRS on day 2 and grade 4 ICANS on day 5, requiring treatment with tocilizumab, anakinra, and high-dose corticosteroids	[52]	2025
27	SSc	CD19	6	All 6 patients remained event-free (no progression of lung, heart, renal disease; no treatment re-initiation) during a median follow-up of 487 days; CRS in all patients (grade 0 in 1 patient, grade 1 in 3, and grade 2 in 2); 1 patient (17%) was hospitalized for influenza with bacterial superinfection; median mRSS decreased by 31% (8 points) within 100 days; digital ulcers reduced fourfold within 3 months; hand function improved (Cochin score decreased by 25.9%; grip strength increased by 46.3%; Moberg test time decreased by 36.6%); lung disease extent on CT decreased by a median of 4% due to reduced ground-glass opacities; FVC improved by a median of 195 mL; antinuclear antibodies declined 10-fold; anti-RNA polymerase III abrogated in 1 patient (later reappeared at low level); anti-topoisomerase I antibodies ↓>90%; EUSTAR-AI declined by a median of 47.5% (2.1 points), with 4 out of 6 patients <2.5 at latest follow-up; patient-reported disability (HAQ-DI) remained stable or decreased by up to 100% (1.75 points)	[53]	2025

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TABLE 1 (CONT.)

Pre-clinical and clinical studies of autologous CAR-T cell therapy for the treatment of autoimmune diseases.

Study	Disease	CAR-T target	No of subjects	Outcome/result	Reference/ partner	Year
28	SSc	CD19	5	CAR-T cells expanded robustly in all patients, with B-cell depletion by day +7; B cells were detectable again at 3 months in 3 patients; skin involvement improved in 3 patients (mRSS decrease), and body weight increased by 12 kg in 1 patient; lung function improved in 3 patients (FVC and DLCO); Scl70 autoantibodies temporarily became negative in 2 patients; RNA polymerase III autoantibodies declined in 2 patients; immunoglobulin (IgG and IgM) levels decreased in all patients post-infusion, but returned to baseline over time; all discharged patients were free of immunosuppressive therapy; 4 patients experienced minimal adverse effects (grade 1 CRS in 3 patients); 1 patient experienced grade 1 CRS followed by prolonged cytopenias (neutropenia grade 4, thrombocytopenia grade 3, anemia grade 2) and subsequently developed secondary HLH with viral reactivation (HSV-1 and CMV), leading to gastrointestinal bleeding and death on day 74; interpretation of fatality: The death was deemed related to CAR-T cell therapy due to excessive CAR-T expansion triggering secondary HLH, but pre-existing risk factors—including advanced age, severe organ dysfunction, prolonged cytopenias, and a TET2 mutation—were also believed to have contributed to the outcome	[54]	2025
29	Refractory LN	BCMA	7	7 patients were followed up for a median of 9 months; median SLEDAI-2K scores dropped from 18 at baseline to 0 at the last follow-up, and 5 out of 7 patients achieved complete remission by 9 months (according to DORIS criteria); all patients reached LLDAS by 6 months post-infusion; proteinuria and renal function improved significantly, and a repeat biopsy in 1 patient confirmed reduced immune complex deposition; peripheral B cells were fully depleted within the first month and had mostly recovered by 3 months; safety was favorable: 1 case of grade 1 CRS; no ICANS; no severe infections reported; hypogammaglobulinemia occurred in all patients, with 5 requiring IVIG; cytopenias related to lymphodepletion were common, but all resolved within 4 weeks	[55]; Shenzhen Pregene Biopharma Company, Ltd	2025

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TABLE 2

Pooled patient outcome following autologous CAR-T cell therapy across autoimmune disease types.

Autoimmune disease	Remission*	Near remission†	Major symptom/clinical improvement‡	No improvement/ worsened condition§
APLS + DLBCL	1	0	0	0
SLE	9	0	1	0
MG	0	3	10	0
Myositis and ILD associated with ASS	0	1	0	0
NMOSD	0	11	1	0
ASS	1	0	0	0
Stiff person syndrome	0	0	1	0
MS	0	0	1	1
B cell lymphoma in a patient with SLE + APLS	1	0	0	0
Concomitant MG + LEMS	0	0	2	0
IIM	0	0	3	0
SSc	2	0	1	0
Juvenile dermatomyositis	0	0	1	0
Patient with RA + DLBCL	1	0	0	0
SLE with LN	12	0	1	0
IMNM	0	0	1	0
LEMS	0	0	1	0
RA	1	0	0	0
Refractory LN	5	2	0	0

*Patients explicitly reported as having achieved complete remission. †Patients meeting criteria for near remission such as those classified as achieving LLDAS or equivalent measures in other studies. ‡Patients meeting criteria for near remission such as those classified as achieving LLDAS or equivalent measures in other studies. §Patients whose symptoms or clinical status remained unchanged or deteriorated.

of follow-up, generally of up to 1 year after therapy. Consequently, there is limited knowledge of lasting treatment benefits, delayed side effects, and long-term risks associated with CAR-T cell immunotherapy in this context. Accordingly, there is a need for larger, more representative trials with control groups to improve consistency and validity of results and allow for comparisons between trials. Longer follow-up periods in these trials will improve insight into the true therapeutic impact, durability, and safety of CAR-T cell therapy for autoimmune disease.

Beyond these general limitations, interpretation across studies is further constrained by significant heterogeneity in both trial design and reporting. For example, some studies used fresh CAR-T cells while others used cryopreserved products, but outcomes were not reported in a consistent manner that would allow for meaningful comparison of their relative clinical benefit. Similarly, although patients with systemic lupus erythematosus (SLE) made up most patients in the above studies, available data do not allow for strong comparisons between SLE

and other autoimmune diseases such as myasthenia gravis. Variations in dosing, lymphodepleting regimens, and CAR constructs (including different generations) further complicate cross-study analysis. Consequently, it is unclear whether certain diseases, patient subgroups (e.g., adult vs pediatric), or CAR-T characteristics provide greater therapeutic benefit. To address this, larger, standardized, and controlled studies are required that ideally directly compare these parameters.

Our review differs from existing publications in several ways. For instance, Sayed *et al.* conducted a systematic review focusing exclusively on SLE, and Cinigreddy *et al.* provided a broader overview of systemic autoimmune diseases but included only nine descriptive studies [56,57]. In contrast, we have sought to integrate both pre-clinical and clinical data across multiple autoimmune conditions from 29 reports, with a specific emphasis on autologous CAR-T cell therapy. Additionally, by organizing safety outcomes and highlighting the need for improved trial designs and manufacturing processes, this review provides a more comprehensive and clinically relevant foundation for future translation of CAR-T therapy in autoimmune diseases.

Despite the high remission rates observed, this systematic review identified two primary groups of adverse effects: CRS and cytopenias attributed to lymphodepleting chemotherapy. To reduce the risk of CRS, emerging technologies such as self-regulating CAR-T cells may be considered [58]. In pre-clinical studies, this approach has been shown to manage excessive cytokine production through an integrated system that controls their activation based on cytokine levels. This mechanism can help prevent CAR-T cell overactivation, thereby reducing the risk of severe CRS and improving the safety profile of these therapies [58]. It is possible that lymphodepletion in autoimmune patients may not need to be as intensive as in the cancer setting, as studies have suggested that CAR-T cell therapy remains effective in autoimmunity even with a higher number of lymphocytes following less intensive lymphodepletion [59]. It is important to note also that lymphodepleting chemotherapy alone cannot fully explain the long-term beneficial effects of CAR-T cell therapy, as study 17 shows that T cells recover within 3 weeks following lymphodepletion [43]. There is also the possibility of eliminating the need for lymphodepletion in these patients, for example, by transient activation of STAT5 signaling in the infused T cells [60]. Potentially, this could avoid significant side effects associated with lymphodepletion, offering a safer and more efficient pathway for autologous CAR-T cell therapies.

Future implementation of CAR-T cell therapy for autoimmune disease will present several challenges. Manufacture of autologous CAR-T cell therapies under GMP (good manufacturing process) is a highly complex and labor-intensive process that requires highly trained personnel. These therapies are uniquely applicable to the donor of the starting material, meaning that a single batch of drug only treats one patient. Moreover, manufacturing processes are poorly automated, which increases the risk of potential human error and makes it harder to scale up and out for widespread use [61]. Furthermore, the time between initiating production to administration of the therapy (vein-to-vein time) is often well over 9 days [62]. This leads to delayed treatment and, consequently, may require the use of bridging therapies. Manufacturing costs of CAR-T cell products can be as much as $350,000–500,000 due to the use of expensive GMP-grade consumables and sophisticated equipment required for production. This limits the economic sustainability of the therapy and reduces accessibility, especially in remote areas [63].

In most cases, cryopreservation of CAR-T cells is used to allow transport of

these labile products from centralized manufacturing facilities to sites of patient treatment. However, this may compromise viable cell count and biological activity of the drug product [64,58]. Reassuringly, studies have shown that sufficient expansion of cryopreserved CAR-T cells can be achieved with little difference in cytotoxicity compared to fresh infusions [65,66].

There are new approaches that can streamline the complex and time-consuming manufacturing process of autologous CAR-T cells. Illustrating this, the T-Charge™ manufacturing platform developed by Novartis reduces culture time to less than 2 days while preserving stem-like T cells. This not only shortens and simplifies production but has also shown enhanced expansion capabilities and improved efficacy [67]. The CliniMACS Prodigy system has demonstrated simplified CAR-T cell production using an automated approach while maintaining comparable efficiency, quality, and yields [68]. Similarly, the Cocoon platform developed by Lonza increases the automated nature of the manufacturing process [69]. In addition, the Cell Shuttle is a compact, fully automated system that integrates all steps involved in CAR-T manufacture and enables the simultaneous production of multiple patient doses, reducing labor requirements, facility needs, and costs [70]. These approaches, along with other manufacturing systems - including wave-mixed (Xuri™ W25), stirred tank (e.g., Eppendorf BioFlo® 320, XDR-10), semi-permeable membrane (e.g., G-Rex®, Sefia™ Expansion System) and vertical wheel bioreactors (e.g. PBS)—could enable either centralized or decentralized CAR-T cell production at hospital sites, reducing complexity, manufacturing time and potential cost, while making the therapy more accessible to a wider population.

Beyond optimizing *ex vivo* manufacturing platforms, entirely new strategies are being explored. One exciting emerging approach is the *in vivo* generation of CAR-T cells. Here, a targeted gene delivery system, such as a lipid nanoparticle or lentiviral vector, is used to deliver the CAR gene into T cells *in vivo*, obviating the need for complex *ex vivo* manufacture or lymphodepletion. Preclinical and early clinical studies have demonstrated proof-of-concept for the efficacy and safety of this approach [71,72]. By avoiding several complex manufacturing steps, this method could shorten production times, reduce costs, and improve accessibility. However, risks of off-target genetic modification, inflammatory reactions, and uncertain long-term persistence must be addressed through further clinical research.

Another important challenge to consider is the shift in clinical delivery systems required to treat patients with autoimmune disease using CAR-T cell therapy. Currently, specialized hematology and oncology centers have both experience and clinical infrastructure required for the administration of CAR-T cell immunotherapy and in the management of toxicities such as CRS and ICANS that arise from this therapy. By contrast, autoimmune diseases are typically managed by rheumatologists using biologics and/or immunosuppressive agents, but not advanced therapeutic medicinal products such as CAR-T cells. Ultimately, close multidisciplinary teamwork will be required to enable effective clinical delivery of these drugs to patients with autoimmune disease.

Recently, there has been a shift in focus to allogeneic CAR-T cells, which are derived from healthy donors, to deal with some of the issues described above with autologous CAR-T cell manufacturing. These therapies can be produced in large batches as 'off the shelf' products that are readily available for many patients [61]. This bulk production also contributes to a reduced manufacturing cost due to the lack of patient-specific collection and processing [68]. However, there are several disadvantages associated with allogeneic CAR-T cells, most notably

the risk of graft-versus-host disease (GvHD) [73,74]. In the oncology setting, allogeneic CAR-T cells also exhibit reduced persistence and long-term efficacy, potentially due to rejection by the host immune system following recovery from the effects of lymphodepletion [75].

Several other emerging approaches, such as CAR-engineered regulatory T cells and CAR NK cells, are being developed to address the limitations of autologous CAR-T cells for treating autoimmune diseases [76–80]. While these therapies show promise in pre-clinical research, there is limited clinical evidence of their efficacy and long-term persistence compared to autologous CAR-T cells.

CONCLUSIONS

Overall, the development and commercialization of autologous CAR-T cell therapy is clearly an emerging approach for the treatment of autoimmune disease. Clinical data gathered to date demonstrate the principle that this approach can achieve high disease remission rates across a spectrum of autoimmune disorders, accompanied by manageable toxicity. However, sample sizes of studies undertaken to date have been very small. Moreover, none of these studies have used control groups, and the follow-up periods have been too short to be certain of the long-term success of this approach. Therefore, it is imperative that future research includes randomized trials with continuous, long-term follow-up. Assuming future research confirms that autologous CAR-T cell therapy is safe and effective long-term, the commercialization of CAR-T cells using the autologous approach is likely to be feasible. While current manufacturing processes are complex and costly, emerging solutions such as the T-Charge platform and *in vivo* generation of CAR-T cells show promise in reducing manufacturing complexity and scalability. Further research is needed to investigate these innovations in context and practice, ensuring that autologous CAR-T cell therapy becomes more accessible and sustainable for widespread use.

TRANSLATION INSIGHT

Autologous CAR-T cell therapy demonstrates compelling efficacy for several autoimmune diseases, but translation into routine clinical use presents significant scientific and logistical barriers. Firstly, current clinical trials are small, uncontrolled, and short-term, providing limited understanding of long-term safety and remission durability. Hence, larger, randomized trials with longer follow-up are needed to establish consistent therapeutic benefit and regulatory approval.

Manufacturing remains a major hurdle. Conventional manufacturing is highly individualized, labor-intensive, and expensive, limiting scalability. However, new innovations such as the T-Charge platform and automated systems like CliniMACS Prodigy® may reduce manufacturing time and cost while preserving cell quality. Furthermore, *in vivo* CAR-T cell generation offers the possibility of bypassing many of these manufacturing steps entirely, potentially improving accessibility and reducing costs.

Moreover, the primary adverse effects observed with CAR-T therapy are linked to the preparatory lymphodepletion regimen. Strategies aimed at reducing or eliminating the need for lymphodepletion—such as the activation of STAT5 signaling—may significantly attenuate associated toxicities.

Beyond technical advances, healthcare infrastructure must adapt. Administration of CAR-T cells for autoimmune conditions will require new collaborations between rheumatology, hemato-oncology, intensive care, and neurology teams, along with tailored training and support.

From a regulatory perspective, tailored guidelines will be essential to evaluate

CAR-T cell therapy when used beyond oncology. Overall, advancing autologous CAR-T cells from primary experimental success to a feasible, scalable therapy for autoimmune disease will require a combination of further clinical research, advancements in manufacturing, and developments in regulatory frameworks.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Modular iPSC workflow for allogeneic cell therapy applications: from iPSCs to iNKs

Omar Farah

The transition of induced pluripotent stem cell (iPSC)-derived cell therapies from research to clinical applications faces significant barriers, including limited supply of regulatory-compliant reagents, scale-up challenges, and variable differentiation protocols. This article describes modular, xeno-free workflow solutions that address these limitations through systematic integration of compliant reagents, closed-system processing, and standardized differentiation protocols, using natural killer cell generation as a model therapeutic application.

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MANUFACTURING BARRIERS LIMIT NK CELL THERAPY TRANSLATION

Natural killer (NK) cell therapies offer advantages over T cell-based approaches, including potential for allogeneic use without human leukocyte antigen (HLA) matching and reduced risk of cytokine release syndrome (CRS). However, current manufacturing approaches are a limiting factor.

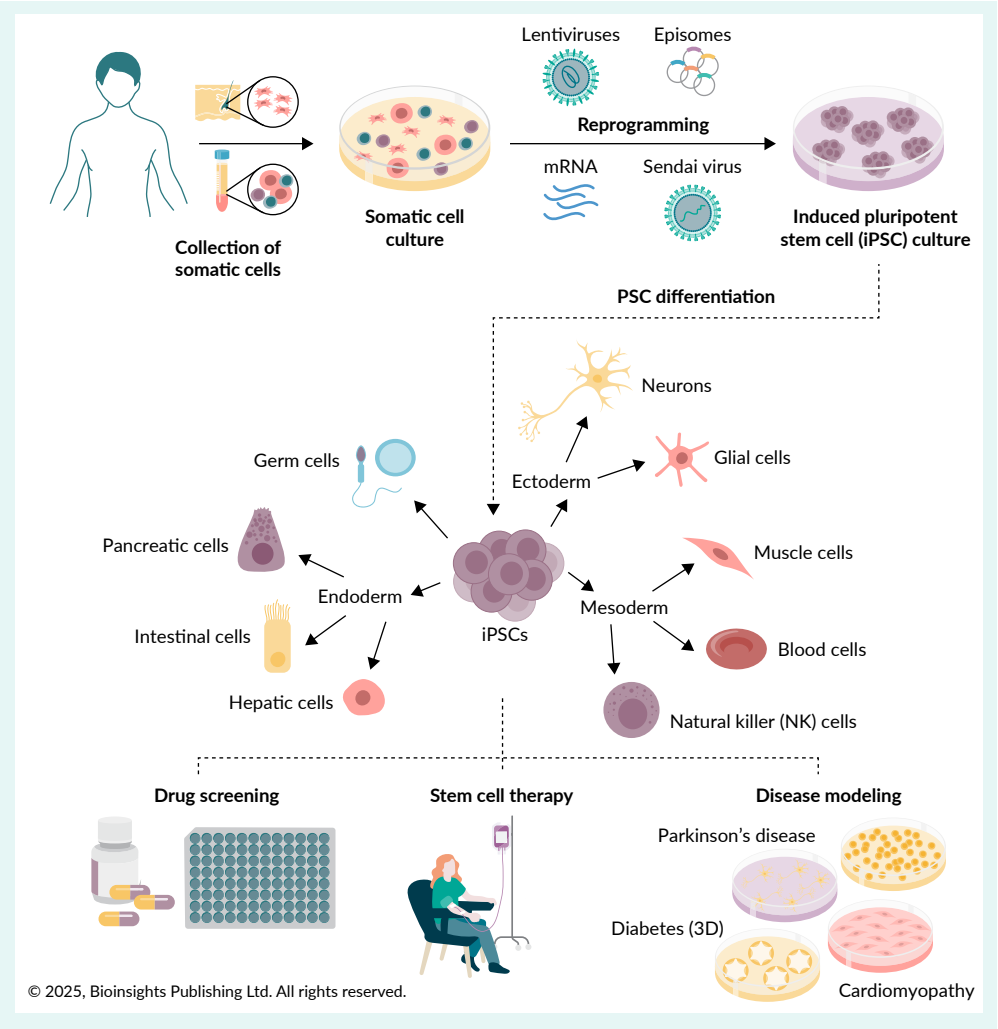
Primary NK cells exhibit substantial donor-to-donor variability in both functional capacity and expansion potential. Individual donors provide differing quantities of NK cells with varying cytotoxic capabilities, creating challenges for standardized therapeutic production. Established NK cell lines provide more consistency but often lack the complete functional repertoire required for therapeutic efficacy.

iPSC-derived NK cells address these manufacturing limitations by providing a standardized cell source. A single, characterized iPSC line can generate multiple therapeutic batches with consistent genetic backgrounds and functional characteristics. This approach enables reproducible manufacturing processes while maintaining the advantages of allogeneic NK cell therapies over autologous cell therapies.

Figure 1 illustrates a modular workflow approach to iPSC-derived NK cell production, integrating clinical-grade components. The workflow progresses from the isolation of peripheral blood mononuclear cells (PBMCs) through iPSC reprogramming, expansion, optional genetic modification, and differentiation into functional NK cells.

FIGURE 1

Modular iPSC workflow for allogeneic NK cell manufacturing.



Systematic integration of clinical-grade components from PBMC isolation through differentiation to functional NK cells. © 2025, BioInsights Publishing Ltd. All rights reserved.

Each step utilizes regulatory-compliant reagents and validated processes designed for clinical manufacturing environments.

CLINICAL-GRADE REPROGRAMMING REDUCES TRANSFORMATION RISK

Traditional iPSC reprogramming methods utilize c-Myc among the reprogramming factors, which poses regulatory challenges for clinical applications due to its oncogenic potential. The CTS™ CytoTune™ 2.1 Sendai Reprogramming Kit addresses this limitation by substituting L-Myc for c-Myc in the reprogramming cocktail. This modification reduces transformation risk while maintaining reprogramming efficiency.

PBMC isolation was performed using the CTS™ Rotea™ Counterflow Centrifugation System in a closed, modular format. The isolated PBMCs underwent reprogramming using the CTS CytoTune 2.1 kit, which supports reprogramming of multiple cell types, including PBMCs. The protocol utilizes xeno-free media throughout the process: CTS™ StemPro™-34 Medium supports PBMC populations during the initial

phases, while CTS™ Essential 8™ or CTS™ StemFlex™ Media maintain pluripotent stem cell cultures post-reprogramming.

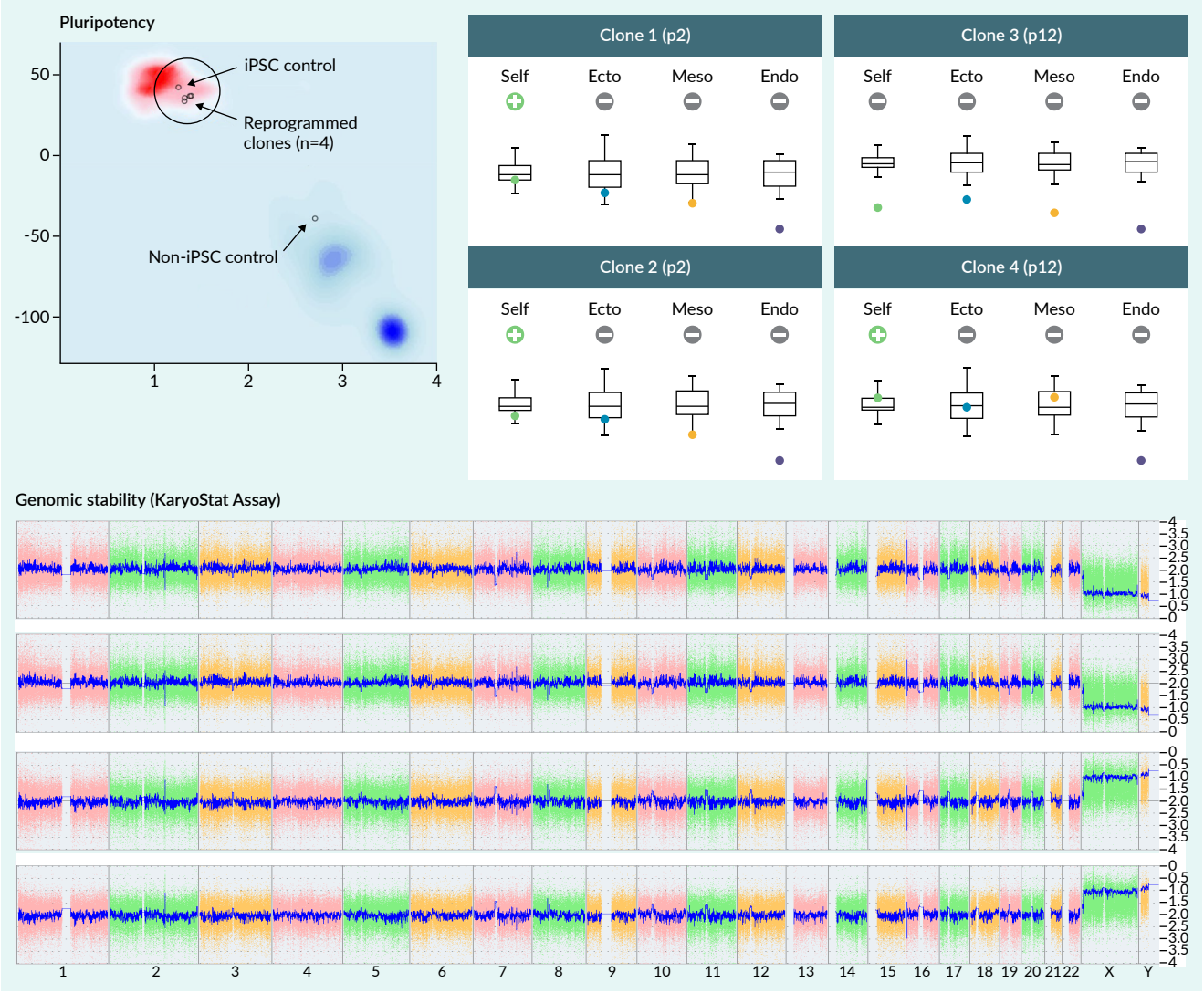
A comparative analysis demonstrated that CTS StemPro-34 Medium achieved a reprogramming efficiency of 0.025% compared to 0.018% with research-grade formulations. Following reprogramming, multiple clones were isolated and characterized using transcriptome-wide analysis via PluriTest™ arrays and targeted qPCR through PSC Scorecard assays to examine global gene expression patterns.

Comprehensive characterization of four representative clones shows the reproducibility of this approach (Figure 2). In this study, two clones were selected manually and characterized at passage 2, while two additional clones underwent FACS sorting using the Invitrogen™ Bigfoot™ Spectral Cell Sorter and were characterized at passage 12. All clones were then adapted to CTS StemFlex Medium for continued expansion.

Karyotype analysis using SNP array-based methods confirmed genomic stability across all tested clones. PluriTest arrays

FIGURE 2

Characterization of PBMC-derived iPSC clones.



Four representative clones demonstrate reproducible pluripotency and genomic stability following L-Myc reprogramming.

indicated that all four reprogrammed clones maintained normal karyotypes and pluripotent transcriptome signatures. Scorecard analysis verified the absence of germ layer markers in all four clones, confirming maintenance of the pluripotent state.

CLOSED-SYSTEM PROCESSING ENABLES CLINICAL MANUFACTURING SCALABILITY

iPSC expansion for therapeutic applications requires processing of cell numbers significantly larger than those typically used in research and discovery efforts. Therapeutic doses demand 10^8–10^9 cells per patient, representing an approximate 1,000-fold increase over research quantities. Manual processing at these scales introduces contamination risks and operator variability that are incompatible with the requirements of clinical manufacturing.

The CTS Rotea Counterflow Centrifugation System addresses these scalability requirements through closed-system processing. The technology utilizes fluid dynamics principles to create a fluidized cell bed by balancing centrifugal force against a counterflowing buffer. This enables cell concentration and washing without environmental exposure during processing.

In the study, process validation was compared between manual and automated approaches using equivalent cell numbers. Both methods maintained similar transcriptomic patterns and pluripotency marker expression. Automated processing reduced processing time from 4–6 hours to 15–20 minutes for billion-cell batches while eliminating operator-dependent variability.

The modular expansion approach progressed systematically from small- to large-scale formats (Figure 3). Initial cultures in 6-well plates expanded to 10-layer cell factory systems, yielding 1–3 billion cells after three weeks of culture. The CTS Rotea system processed the harvested cells through wash and concentrate protocols.

Suspension culture utilized CTS™ StemScale™ Medium to support spheroid formation while maintaining pluripotency markers. The transition from adherent to suspension format enabled expansion in bioreactor systems. Spheroid cultures demonstrated a consistent 8- to 10-fold expansion per passage across multiple cell lines.

Long-term culture validation was extended to 30 consecutive passages over six months. Cells maintained consistent spheroid morphology as observed at passages 15 and 30. Flow cytometric analysis revealed >90% expression of pluripotency markers (OCT4 and NANOG) throughout the culture period. Karyotype analysis detected no chromosomal abnormalities over the 30-passage duration.

NON-VIRAL GENE EDITING APPROACH ENABLES RAPID MODIFICATION CYCLES

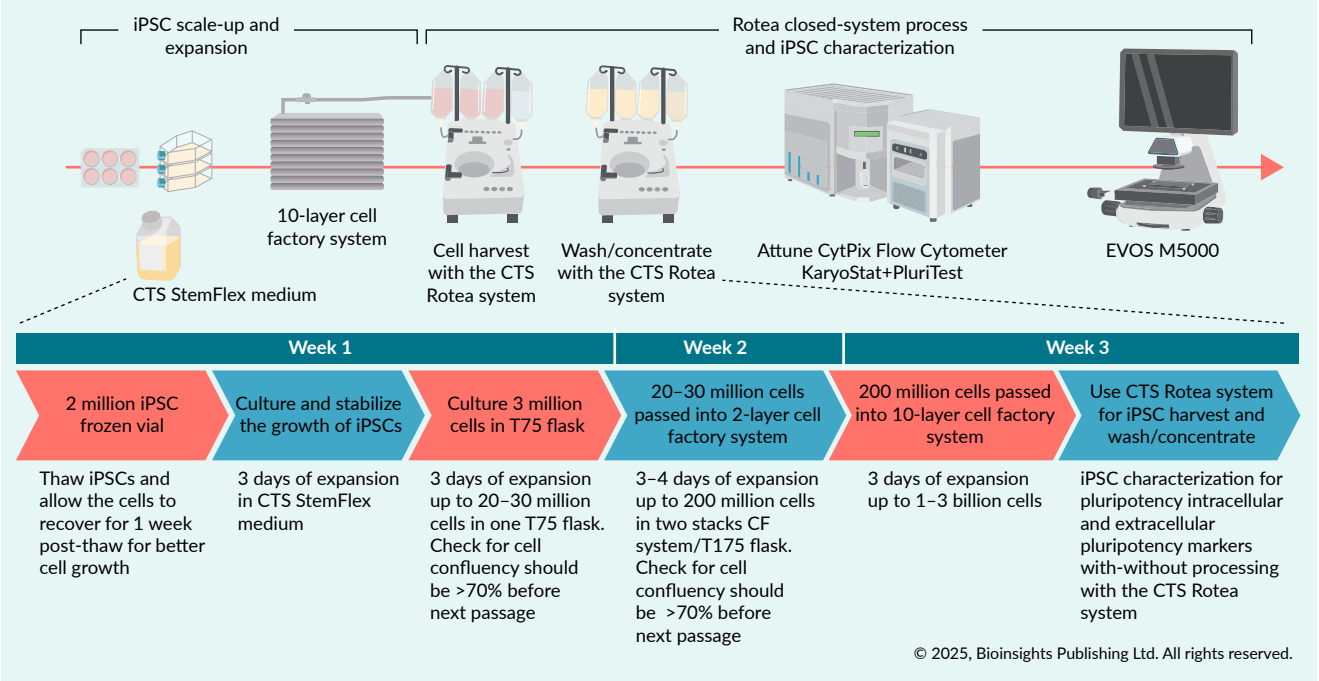
Viral vector production for iPSC modification involves complex manufacturing requirements, extended development timelines, and comprehensive safety testing protocols. The process typically requires 3–6 months for vector modifications, while replication-competent virus testing adds additional time and cost considerations.

Electroporation-based delivery offers an alternative approach through the direct introduction of ribonucleoprotein complexes. The method applies controlled electrical pulses to create transient membrane pores, allowing the entry of molecular complexes while preserving cell viability. This approach eliminates the need for viral vector production and enables modification testing within days of design completion.

In a recent study, the electroporation optimization process focused on balancing membrane permeabilization with cell survival. Parameter optimization identified critical settings: pulse voltage (1,350 V) for sufficient field strength, reduced pulse

FIGURE 3

Closed-system processing enabled billion-cell scale expansion.



CTS Rotea system reduced processing time from 4–6 hours to 15–20 minutes while maintaining transcriptomic consistency.

width (20 ms) to minimize cellular damage, and specialized buffer formulations to reduce cellular stress. Using CIITA gene targeting as a model system, optimized conditions achieved an editing efficiency of >50% while maintaining cell viability of >80%. Recovery analysis showed normal growth patterns within 72 hours post-electroporation, indicating that the conditions helped avoid extended recovery periods that could impact manufacturing workflows.

Single-cell clonal expansion presents technical challenges due to the isolation stress that occurs when cells are separated from their supporting intercellular contacts. Enhanced media formulations address this challenge by providing survival factors and antioxidant systems that compensate for lost intercellular communication while maintaining pluripotency signaling. In the study, this approach achieved 35% clonal expansion efficiency, representing a 3-fold improvement over typical protocols.

CHIMERIC ANTIGEN RECEPTOR INTEGRATION DEMONSTRATED TARGETED INSERTION AT THE CD38 LOCUS

Chimeric antigen receptor (CAR) integration utilized targeted insertion at the CD38 locus through clustered regularly interspaced short palindromic repeats (CRISPR)-mediated cutting combined with double-stranded DNA templates. The CD38 locus was selected because it is dispensable for NK cell function while providing an actively transcribed genomic location for CAR expression.

The molecular delivery strategy involved the simultaneous introduction of CRISPR components and a DNA template: a Cas9 protein complexed with guide RNA targeting CD38, plus double-stranded DNA carrying the anti-mesothelin CAR construct flanked by homology arms matching the CD38 sequence (Figure 4).

Characterization of modified clones confirmed preservation of cellular characteristics following targeted integration. All tested clones maintained normal karyotypes and pluripotency signatures, indicating that CD38 disruption and CAR insertion did not compromise basic cellular functions. The integration process preserved the differentiation potential, with modified iPSCs successfully generating functional NK cells that expressed normal surface markers.

Flow cytometric analysis comparing unedited control cells with CAR-modified cells demonstrated successful genetic modification while preserving differentiation capacity. Unedited iPSCs differentiated into functional NK cells without CAR expression, while CAR-modified iPSC pools maintained differentiation capacity with approximately 20% of cells expressing anti-mesothelin CAR alongside standard NK markers (CD45, CD56, CD16).

The 20% CAR integration efficiency represented a successful Proof of Concept that can be further enhanced through template design optimization or selection strategies. Functional validation demonstrated that CAR-positive NK cells retained their natural cytotoxic pathways while exhibiting enhanced responses to mesothelin-expressing targets.

STANDARDIZED DIFFERENTIATION PROTOCOLS GENERATED FUNCTIONAL NK CELLS

NK cell differentiation from iPSCs has been hindered by protocol variability between laboratories and batches, which is attributed to undefined media components, imprecise cytokine concentrations, variable culture conditions, and a lack of standardized quality control checkpoints.

A systematic 38-day protocol addressed variability through defined culture conditions and standardized reagent concentrations (Figure 5). The protocol progression followed hematopoietic stem cell development: initial hematopoietic specification using CTS StemPro-34 medium with SCF (100 ng/mL), IL-3 (10 ng/mL), and FLT3-L (10 ng/mL), followed by NK specification using CTS™ NK-Xpander™ Medium with IL-15 (10 ng/mL) and IL-2 (100 U/mL).

Flow cytometric analysis demonstrated consistent progression: hematopoietic progenitors emerged by day 9 (57.2% $CD34^+$, 41.8% $CD90^+$), followed by NK specification by day 21 (48.4% $CD56^+/CD3^-$), and then progressed to mature NK cells by day 38 (62.2% $CD56^+$ with enhanced CD16 co-expression).

Post-differentiation expansion achieved 90% NK cell purity, accompanied by a substantial expansion of the cell population. The process generated ratios up to 10 iNK cells/starting iPSC, providing relevant yields for therapeutic applications. Functional validation during expansion demonstrated the maintenance of cytotoxic potential, along with the expression of appropriate activation markers and responses to target cell stimulation.

FUNCTIONAL VALIDATION DEMONSTRATED THERAPEUTIC POTENTIAL

Therapeutic NK cell validation requires demonstration of cytotoxic activity against physiologically relevant tumor targets. The validation approach taken here utilized patient-derived colorectal tumor organoids cultured in OncoPro™ Tumoroid Medium. These 3D cultures maintained tumor architecture and cellular interactions absent in monolayer systems.

The co-culture assay enabled quantitative analysis through real-time monitoring: GFP-labeled tumor organoids provided continuous measurement of viable tumor mass, while caspase 3/7 activation indicated apoptotic cell death mechanisms (Figure 6).

Co-culture assays with patient-derived organoids demonstrated dose-dependent killing across effector-to-target

ratios ranging from 0.625:1 to 10:1. The progressive decrease in tumor cell GFP signal, accompanied by increased caspase 3/7 activation, confirmed the apoptotic cell death mechanisms. Kinetic analysis showed cytotoxic onset within 4–6 hours of co-culture, indicating rapid NK cell activation and target recognition.

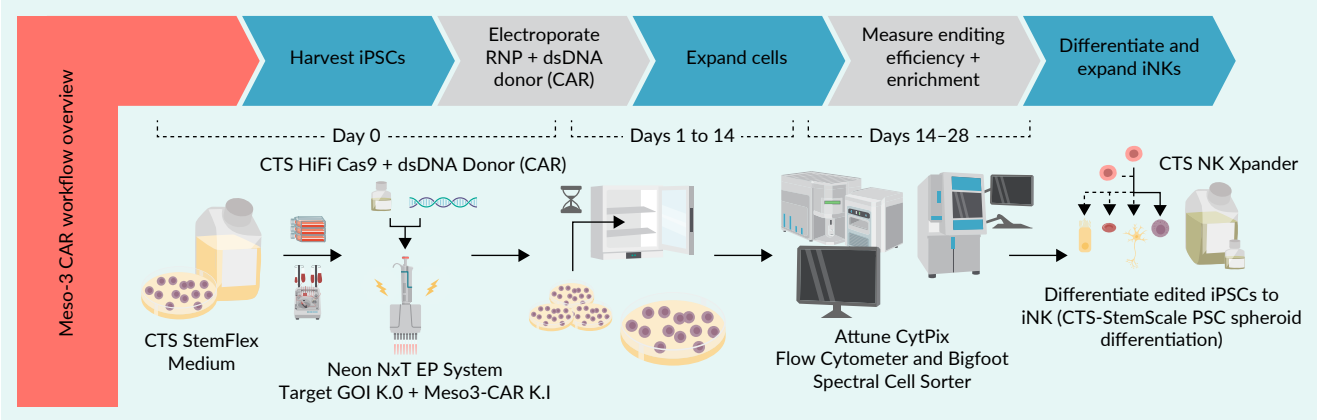
The quantitative dose-response relationship enabled the prediction of therapeutic doses: 50% tumor cell killing requires an approximately 2.5:1 effector-to-target ratio, while 80% killing requires a 5:1 ratio. Cryopreservation studies evaluated manufacturing flexibility requirements. iPSC-derived NK cells maintained >85% viability with continued expansion over 14-day culture periods following cryopreservation and recovery.

MANUFACTURING WORKFLOW DEMONSTRATED CLINICAL FEASIBILITY

Manufacturing validation demonstrated that production capabilities met clinical

FIGURE 4

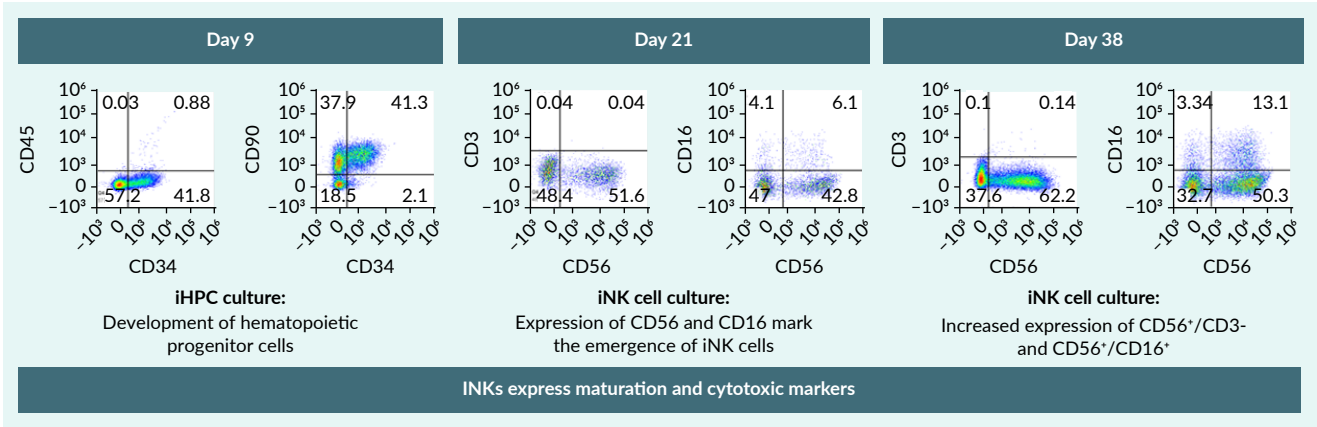
CAR integration workflow and differentiation outcomes.



CRISPR-mediated CAR insertion at the CD38 locus with 20% of differentiated NK cells expressing anti-mesothelin CAR.

FIGURE 5

NK cell differentiation progression over 38 days.



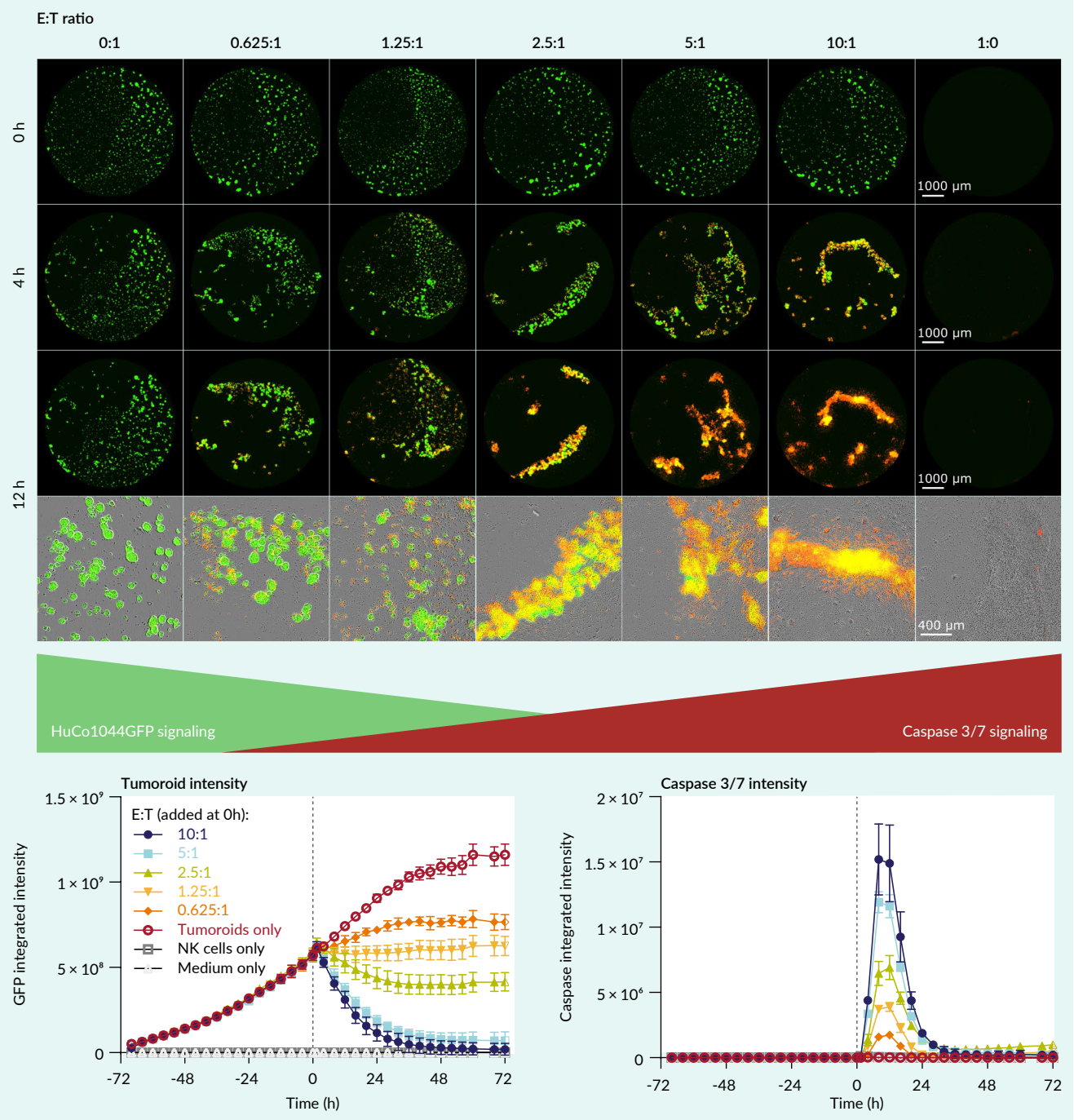
Reproducible marker progression from hematopoietic progenitors (day 9) to mature NK cells (day 38) achieved 90% purity.

requirements, producing 1–4 billion cells with >80% NK marker expression, as per standardized protocols. These yields provided sufficient material for multiple patient doses from a single production run, enabling economies of scale compared to autologous approaches.

Complete workflows from iPSC thaw to final NK cell product required approximately 6–8 weeks. The timeline breakdown

FIGURE 6

Dose-dependent cytotoxic activity against patient-derived organoids.



Quantitative analysis revealed 50% tumor killing at an effector-to-target ratio of 2.5:1 and 80% killing at a ratio of 5:1.

included iPSC expansion and characterization, NK differentiation, and expansion/formulation. Parallel processing of multiple batches further reduced the impact on the per-dose timeline.

Quality control integration throughout the workflow ensured compliance with regulatory requirements for real-time release testing. Standardized assays monitored pluripotency markers, differentiation progression, genomic stability, and functional activity at defined checkpoints, enabling early intervention when parameters deviated from specifications.

The analytical framework included flow cytometric analysis for marker expression, genomic stability assessment through karyotype analysis, functional validation through cytotoxicity assays, sterility testing, and endotoxin quantification. The modular design enabled phased regulatory submissions, while infrastructure design enabled expansion to commercial scales.

SUMMARY

This integrated workflow addressed manufacturing barriers in iPSC-derived NK cell production by systematically implementing clinical-grade components. CTS CytoTune reprogramming enabled the generation of regulatory-compliant iPSCs while maintaining reprogramming efficiency equivalent to that of research methods. Closed-system processing enabled scalable cell production through automated handling that reduced both contamination risks and operator variability.

Non-viral gene editing achieved >50% efficiency through optimized electroporation protocols, eliminating the need for viral vector production while enabling rapid modification cycles. Targeted CAR integration demonstrated successful genetic modification while preserving NK cell differentiation capacity.

Standardized differentiation protocols generated NK cells with >90% purity through a defined 38-day process with reproducible marker progression. Functional validation using patient-derived tumor organoids confirmed cytotoxic activity with dose-dependent killing kinetics, making it suitable for predicting therapeutic doses. Manufacturing integration demonstrated clinical-scale production capabilities supporting regulatory requirements for clinical translation.

Q&A

Michael Akenhead (left), **Abigail Harris Becker** (center left), **Carl Dargitz** (center right), **Omar Farah** (right)

Q Abigail, is the formulation different for the new CTS StemPro-34 Medium?

AHB The formulation is the same, but we did optimize the concentration of most of the components in the supplement. We had to make a few minor changes, switching to recombinant protein and a synthetic component. While the

overall components within the supplement remain the same, we observed improved performance solely due to optimizing the supplement concentrations.

Q How does the performance compare between RUO media and the CTS StemPro-34 Medium?

AHB We find that in a number of applications, we see improved performance, especially in terms of expansion. We also see equivalent or improved differentiation capacity when going from iPSCs to iHSCs.

Q What types of cells have been tested using the CTS StemPro-34?

AHB In addition to the iPSC-derived HSCs that Omar discussed, we have also used this medium with primary HSCs from bone marrow, cord blood, and mobilized peripheral blood.

Q Michael, can PSCs be scaled up in the CTS StemFlex Medium?

MA We don't recommend scaling up in CTS StemFlex because that medium was designed for adherent culture, whereas CTS StemScale was designed for suspension culture. If you plan to scale up to large cell quantities, we recommend starting initially in adherent culture with CTS StemFlex and then switching to CTS StemScale for your scaling work in suspension.

You can transition directly from CTS StemFlex into CTS StemScale - give the spheroids a couple of passages to adapt to the switch from adherent to suspension culture.

Q Do cells grown in the CTS StemFlex Medium maintain trilineage differentiation potential?

MA Yes, they do.

Q Does the CTS StemScale Medium support gene editing of PSC cells?

MA We haven't looked into gene editing using CTS StemScale as yet. If you are looking to do any gene-editing workflows, we would recommend taking those that were expanded in 3D back into 2D using the CTS StemFlex Medium.

Q How does performance compare between the RUO and the CTS versions of the StemFlex and StemScale Media?

MA For the StemFlex media, we observe equivalent performance between the RUO and CTS versions in terms of both growth and maintenance of pluripotency. For StemScale, there is an approximate 24-hour difference in growth between the RUO and the CTS versions. For CTS StemScale, you will require a little extra time to grow the spheroids in order to achieve the same cell yields. However, in terms of maintaining pluripotency, the two media perform similarly.

Q Omar, is the process and workflow you described applicable to CAR-T cell production?

OF Essentially, the workflow steps are the same. There will be differences in the media reagents and protocols regarding the tail end of differentiation, but the concepts can generally be carried forward to CAR-T.

Q At what passage was iPSC characterization done during the expansion phase following reprogramming?

OF Passage 10, but we actually have ongoing work for longer-term characterization that should be completed soon. Stay tuned for more information on that.

Q Did you compare electroporation against LNPs and lentiviral vectors for gene engineering?

OF We haven't conducted a side-by-side study in this context, but that being said, we have some internal work where we have used combinatory approaches—for example, viral transduction in combination with electroporation. There are multiple reasons why you would want to take that sort of approach, particularly when it comes to your construct—the size of the construct itself and the payload that you're trying to deliver—and also whether you want to work within the context of screening clones afterwards. We do have some of the data on that, and technically speaking, it can be done—we have demonstrated that before.

Q Can the CTS NK-Xpander support expansion in the presence of feeder cells?

AHB The CTS NK-Xpander Medium was developed specifically to be used in feeder-free systems, and it does help to reduce some of the added work and risk of using a feeder-based system. However, it can also be used with a feeder-based system, if that's what the user prefers.

Q Are larger configurations of the CTS NK-Xpander Medium available?

AHB At the moment, it is available as a 500 mL bottle kit and a 5 L kit in bags. However, in the coming weeks, we will be launching the supplement for the 5 L kit in a bag, so you can sterile weld this supplement bag to the basal media bag. 10 L and 20 L kits will also be available in a similar timeframe.

Q Is the CTS NK-Xpander Medium used on its own for iNK expansion, or are additional components such as feeder cells typically required?

AHB To make the complete media, you have the basal, the supplement, you add human AB serum, and then cytokines of your choice. For the iNK cells, as with the primary iNK, feeder cells are not required.

Q Do you have media solely for the purpose of differentiating iPSCs to hPSCs?

AHB The CTS StemPro-34 media can be used to differentiate iPSCs into the iHSCs.

Q What is your analysis method for spheroid size?

MA For spheroid size, I like to do daily sampling of the bioreactor culture. I remove a sample of approximately 5 mL, transfer it to a well plate, and get some representative images of the spheroids. I can then quickly assess their size using the scale bar of the microscope. If I want to get more in-depth, I'll take it back to the computer and open up ImageJ, but in general, I just quickly visually assess with daily sampling.

Q Carl, how are you able to use the Rotea for both the harvest and wash-and-concentrate steps within the same workflow?

CD The basic principle for performing different types of functions with the Rotea is based on the fact that it's an open architecture system for building protocols. You can essentially take whatever process you would like to perform and adjust the settings on the Rotea in order to do so.

In this case, you are just looking at the specific cells that you're collecting, the concentrations that you are aiming for, and the total volumes that you want to harvest. There are a whole host of considerations there but from a high level, because it's all user-programmable and you can write your own protocols, you can do that for each unit function. Additionally, on our side, we have standard protocols that we share with customers when they want to use it for a specific purpose.

Q What changes need to be made in Rotea protocols to process different cell types?

CD Ultimately, the main principles at play in a counterflow centrifuge like Rotea are the flow rate and the centrifuge force. If you have larger or smaller cells, you adjust the balance of those two in order to capture them and create a fluidized cell bed in the cone. We have tools built into the software that allow you to estimate what the settings should be for a specific size and density of particle or cell.

That gives you a great starting point from which you can empirically determine what gives you the highest cell recoveries. It's a fairly standard process, and again, if you are using a standard cell type like T or NK cells - or iPSCs - we have standard protocols that you can reference.

BIOGRAPHIES

Omar Farah received his doctorate from McGill University, Montreal, QC, Canada, where he studied early embryo lineage specification and development of the female reproductive tract needed for proper embryo-uterine crosstalk. Omar continued his postdoctoral work at the University of California, San Diego, where he focused on using stem cell-based models to study extra-embryonic lineage specification, trophoblast differentiation, and placental development. Currently, Dr Farah is working at Thermo Fisher, managing a team of regenerative medicine field application scientists supporting stem cell, neurobiology, cell and gene therapy workflows.

Omar Farah, Manager, Field Applications, Thermo Fisher Scientific

Carl Dargitz is a Senior Manager in the Cell & Gene Therapy Platforms R&D group developing cell therapy instrumentation at Thermo Fisher Scientific. His team lead the development of the CTS Rotea Counterflow Centrifugation System. Previously, he developed reprogramming and characterization assays for PSCs and immune cells at Thermo Fisher. Before joining Thermo Fisher, he worked at the Salk Institute for Biological Studies at the stem cell core

facility managing the core's reprogramming and characterization services. He received his MS from California Polytechnic State University, San Luis Obispo, CA, USA in Biomedical Engineering with a specialization in stem cell research in 2013.

Carl Dargitz, Senior R&D Manager, Cell & Gene Therapy Platforms, Thermo Fisher Scientific

Mark Kennedy is a Manager in the Cell & Gene Therapy R&D group at Thermo Fisher Scientific. His team is focused on the development of cell culture media, reagents and protocols that support 2D and 3D pluripotent stem cell-based applications. Prior to joining Thermo Fisher, he obtained his PhD from Memorial University, St John's, NL, Canada where he studied cell signaling during early embryonic development. Subsequently, Mark continued his postdoctoral studies at the NIH-National Cancer Institute where he focused on the use of *in vivo* and *in vitro* embryonic stem cell models to understand how different cell lineages are specified during early embryogenesis.

Mark Kennedy, Manager, Thermo Fisher Scientific

Abigail Harris Becker is a Staff Scientist in the Cell & Gene Therapy R&D group at Thermo Fisher Scientific. Her work focuses on development of cell culture media systems for adult stem cells, including hematopoietic stem cells and mesenchymal stromal cells. Previously, she developed a variety of molecular analysis tools for life science applications. Prior to joining Thermo Fisher Scientific she received her MS from Pennsylvania State College of Medicine, Hershey, PA, USA where she conducted research on genetic and epigenetic dysregulation in hematological malignancies.

Abigail Becker, Staff Scientist, Thermo Fisher Scientific

Michael Akenhead is a Staff Scientist in the Cell & Gene Therapy group at Thermo Fisher Scientific. His primary focus is the development of cell culture media to support the growth of stem cells in 3D suspension culture formats. The results of this research led to the development of the Gibco™ CTS™ StemScale™ PSC Suspension Medium. Prior to joining Thermo Fisher, he obtained his PhD in Biomedical Engineering from the University of Kentucky, Lexington, KY, USA where he studied the mechanobiology of neutrophils and its effects on their immune response.

Michael Akenhead, Staff Scientist, Thermo Fisher Scientific

AUTHORSHIP & CONFLICT OF INTEREST

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ThermoFisher SCIENTIFIC

For more information about the solutions discussed in this article please visit https://www.thermofisher.com/us/en/home/clinical/cell-gene-therapy/cell-therapy/cell-therapy-systems/ipsc-derived-car-nk-cell-therapy-solutions.html

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Stem cell research

Enabling your pluripotent stem cell therapy research

Translate your research to the clinic with CTS iPSC media and reagents

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Advancing your induced pluripotent stem cell (iPSC) therapy research to clinical applications requires careful material selection, as the quality of starting materials can significantly impact the properties of your final stem cell therapy product.

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- Designed to meet the latest guidance for ancillary materials for cell and gene therapy (CGT) (USP <1043>,* ISO 20399, EP 5.2.12.)
- Products manufactured under global quality standards (QMS ISO 9001 or 13485)
- Over 30 years of GMP-compliant manufacturing

Testing and documentation

- Extensive qualification and testing of critical raw materials
- Final product testing (e.g., mycoplasma, endotoxin, sterility)
- Types of traceability documentation: CoO, CoA, SDS

Dedicated regulatory and quality control teams

- Types of specialized documentation include Drug Master File, Regulatory Support File, validation, and regulatory agency letters
- Products used in over 200 clinical trials and commercialized therapies
- Global CGT regulatory affairs teams with regional expertise

* CTS products are designed to meet USP <1043>, ancillary material responsibilities for cell, gene, and tissue-engineered products, under a robust quality management system certified to ISO 9001 or 13485. All aspects of USP <1043> are the responsibility of the end user to assess. We are dedicated to supporting our customers' clinical translation.

iPSC manufacturing workflow

Reprogramming

Cell culture media
Gibco™ CTS™ KnockOut™ SR XenoFree Medium

Cell reprogramming
- Invitrogen™ CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit
- Gibco™ CTS™ Essential 8™ Medium
- Gibco™ CTS™ StemPro™ -34 XenoFree Serum-Free Medium
- Gibco™ CTS™ DPBS
- Thermo Scientific™ Herasafe™ 2030i Biological Safety Cabinet CTS™ Series – EN12469

Cell engineering

Closed cell processing
Gibco™ CTS™ Rotea™ Counterflow Centrifugation System

Nonviral platform
- Gibco™ CTS™ Xenon™ Electroporation System
- Gibco™ CTS™ TrueCut Cas9 Protein
- Gibco™ CTS™ HiFi Cas9 Protein

Lentiviral production system
Gibco™ CTS™ LV-MAX™ Production Medium

Cell culture media and reagents
- Gibco™ CTS™ StemFlex™ Medium
- Gibco™ CTS™ Essential 8™ Medium
- Gibco™ CTS™ Vitronectin (VTN-N) Recombinant Human Protein, truncated

Cell expansion

Cell culture media and supplements
- Gibco™ CTS™ StemScale™ PSC Suspension Medium
- CTS StemFlex Medium
- CTS Essential 8 Medium
- CTS Vitronectin (VTN-N) Recombinant Human Protein

Dissociation reagents
- Gibco™ CTS™ Versene™ Solution
- Gibco™ CTS™ TrypLE™ Select Enzyme
- Gibco™ CTS™ DPBS, without $CaCl_2$ and $MgCl_2$

Cell culture plastics
- Thermo Scientific™ Nunc™ TripleFlask™ Treated Cell Culture Flask
- Thermo Scientific™ Nunc™ Cell Factory systems

Fit-for-purpose lab equipment
Thermo Scientific™ Sorvall™ X4 Pro centrifuges

CO_2 incubators
Thermo Scientific™ Heracell™ Vios 160i CR CO_2 Incubator

Banking and recovery

Cryopreservation
- Gibco™ CTS™ PSC Cryopreservation Kit
- Gibco™ CTS™ PSC Cryomedium

Cell recovery
Gibco™ CTS™ RevitaCell™ Supplement

Differentiation

Cell culture media and reagents
- Gibco™ CTS™ Essential 6 Medium
- CTS KnockOut SR XenoFree Medium
- Gibco™ CTS™ KnockOut™ DMEM
- Gibco™ CTS™ N-2 Supplement
- Gibco™ CTS™ B-27™ Supplement XenoFree
- CTS StemPro-34 XenoFree Serum-Free Medium

Gibco™ PeproGMP™ cytokines and growth factors

Characterization

Applied Biosystems™ TaqMan™ hPSC Scorecard Panel—for assessing trilineage differentiation potential
Applied Biosystems™ PluriTest™ Assay Service—for testing pluripotency
Applied Biosystems™ KaryoStat™ Assays—for checking genomic stability
Thermo Scientific™ Oncomine™ NGS Assay Service—for detection of genomic variations associated with selected cancer hotspots
Invitrogen™ CellModel™ Services—for stem cell characterization

We support the development of your iPSC therapy from the earliest stages with high-quality research-use-only products. Learn more at thermofisher.com/stemcells.

Ordering information

Product	Cat. No.
CTS B-27 Supplement XenoFree	A5047501
CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit	A34546
CTS DPBS, without calcium chloride, without magnesium chloride	A1285602
CTS Essential 6 Medium	A4238501
CTS Essential 8 Medium	A2656101
CTS HiFi Cas9 Protein	A54223
CTS KnockOut DMEM	A1286101
CTS KnockOut SR XenoFree Medium	12618012
CTS LV-MAX Production Medium	A4124001
CTS N-2 Supplement	A1370701
CTS PSC Cryomedium	A4238801
CTS PSC Cryopreservation Kit	A4239301
CTS RevitaCell Supplement (100X)	A4238401
CTS Rotea Counterflow Centrifugation System	thermofisher.com/rotea

Product	Cat. No.
CTS StemFlex Medium	A5465001
CTS StemPro-34 XenoFree Serum-Free Medium	A6636901
CTS StemScale PSC Suspension Medium	A5869601
CTS TrypLE Select Enzyme	A1285901
CTS Versene Solution	A4239101
CTS Vitronectin (VTN-N) Recombinant Human Protein, truncated	CTS27953
CTS Xenon Electroporation System	thermofisher.com/xenon
Heracell Vios 160i CR CO_2 Incubator	51033770
Herasafe 2030i Biological Safety Cabinet CTS Series - EN12469	51033758
Nunc Cell Factory systems	139446
Nunc TripleFlask Treated Cell Culture Flask	132867
Sorvall X4 Pro centrifuges	75009505
CTS TrueCut Cas9 Protein	A45220
PeproGMP cytokines and growth factors	thermofisher.com/peprogmp

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gibco

Deep phenotypic and cytotoxicity characterization of NK cells cultured with chemically defined additives

Rebecca Nickle, Senior Technical Applications Scientist, BioLegend

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Immunotherapy is transforming cancer treatment by harnessing the body's immune system, with natural killer (NK) cells emerging as promising alternatives to T cells due to their innate cytotoxicity. However, delivering effective NK cell therapies highly depends on the ancillary materials used during *ex vivo* bioprocessing and robust cell characterization. This poster outlines a workflow from NK cell isolation to serum-free culture and cytotoxic analysis, offering a comprehensive view of NK cell function through targeted killing, surface marker profiling, and secreted molecule characterization.

SERUM-FREE NK CELL EXPANSION

In a proprietary study, human peripheral blood mononuclear cells (PBMCs) were isolated from a leukocyte reduction system using Lymphopure™ medium. NK cells were subsequently isolated from PBMCs using MojoSort™ Human NK Cell Isolation Kit and Cell-Vive™ CD Cell Separation Buffer, GMP, followed by culturing in standard sera (fetal bovine and human AB), or a serum substitute optimized for NK cell expansion, along with IL-15, IL-18, and IL-27 cytokines. Results demonstrated that NK cells cultured with 5% Cell-Vive T cell CD Serum Substitute expand significantly more than control groups (Figure 1), showing that optimized, serum-free conditions support robust NK cell expansion, which provides a reliable platform for downstream functional assays and potential therapeutic applications.

NK CELL PHENOTYPE ANALYSIS: SURFACE MARKERS

In another experiment, NK cells were analyzed for surface markers CD56, CD16, NKG2A, and NKG2C after 21 days of culture. Cells cultured in a chemically defined serum substitute exhibited a $CD56^+$ $CD16^+$ $NKG2A^+$ phenotype, characteristic of mature, proinflammatory NK cells (Figure 2). This assay indicates that the culture conditions support the development of highly active NK cells suitable for therapeutic applications.

NK CELL FUNCTIONAL ANALYSIS: CYTOTOXICITY ASSAY

In addition to surface phenotyping, 48-hour culture supernatants were collected and analyzed using LEGENDplex™ Human CD8/NK Panel, detecting cytokines such as IL-6, IL-17A, TNF-α, IFN-γ, perforin, granulysin, and granzymes A/B (Figure 3). NK cells cultured with Cell-Vive T cell CD Serum Substitute showed significantly increased secretion of cytotoxic and proinflammatory molecules. These findings, combined with surface marker analysis, indicate that the serum substitute supports enhanced NK cell activation and function, offering an efficient platform for generating potent NK cells for immunotherapy.

SUMMARY

GMP serum-free media can support robust NK cell expansion and promote a mature phenotype with high expression of CD16, NKp30, CD161, and NKG2A, along with increased proinflammatory cytokine production. The chemically defined composition additionally reduces variability and risks associated with traditional serum, such as human AB, enabling researchers to better explore NK cell biology and advance the development of robust NK cell therapy workflows.

Figure 1. Greater NK cell expansion with Cell-Vive T cell CD Serum Substitute, GMP.

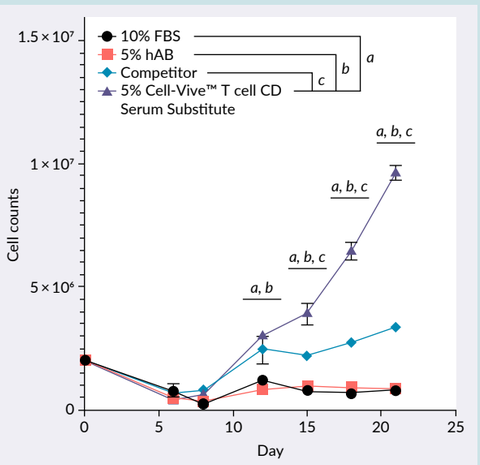


Figure 2. Purified NK cells cultured in Cell-Vive T cell CD Serum Substitute, GMP, exhibited a more mature and proinflammatory phenotype.

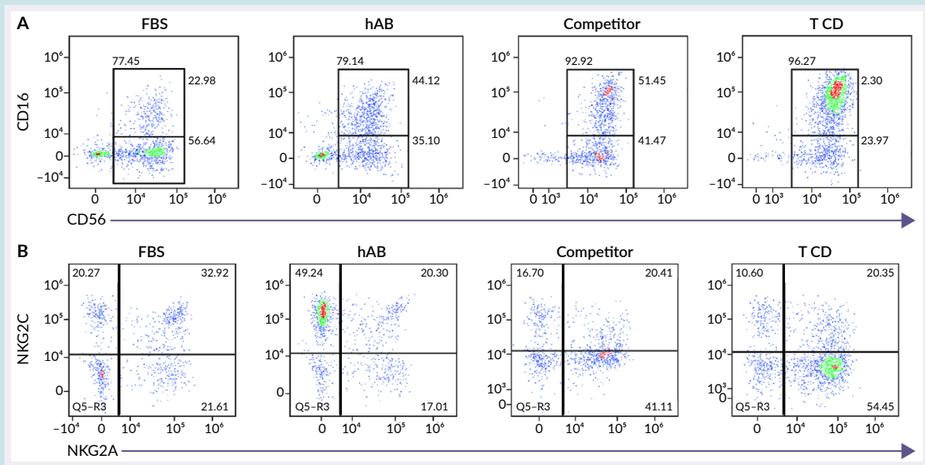
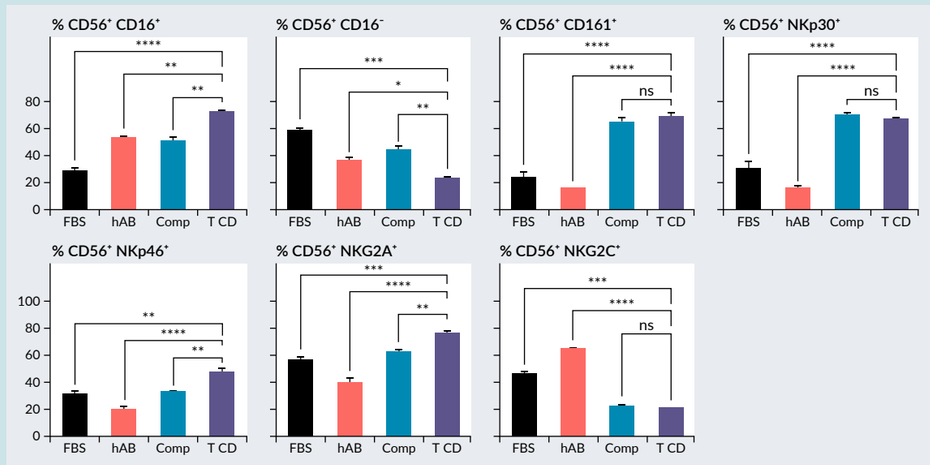


Figure 3. Increased production of cytotoxic and proinflammatory cytokines by NK cells cultured with Cell-Vive T cell CD Serum Substitute, GMP.



Rebecca Nickle PhD is a Senior Technical Applications Scientist at BioLegend, from Revvity, specializing in immunology applications and research support. With her doctoral training in immunology, she serves as a primary technical resource for customers, providing expert consultation on experimental design, technology selection, and troubleshooting across BioLegend's comprehensive product portfolio. Her expertise in advanced techniques, including flow cytometry and CITE-Seq, helps researchers optimize their immunological investigations and multiparameter analyses.

CELL & GENE THERAPY INSIGHTS

Perspectives on quality, compliance, and innovation to accelerate CGT manufacturing

INTERVIEW

"...the integration of automation, standardized protocols, evolving regulatory frameworks, and robust scale-up strategies will be critical..."

Lauren Coyle, Editor, BioInsights, speaks to **Hélène Negre**, Pharmaceutical Affairs Director, CELL*for*CURE, about the manufacturing of ATMPs and how they are undergoing rapid transformations, driven by the need for speed, safety, and scalability. The article shares insights on key challenges in batch release, the importance of cross-functional collaboration, and innovations shaping the future of CGT manufacturing.

Cell & Gene Therapy Insights 2025; 11(8), 1025–1031 · DOI: 10.18609/cgti.2025.117

Q From your experience across clinical and commercial settings, how has the role of the qualified person (QP) evolved in CGT manufacturing, particularly in balancing speed and compliance?

HN The role of the QP has become increasingly critical in CGT as the field has advanced significantly over the past decade. This evolution has been driven by the unique scientific and logistical challenges of advanced therapies, heightening

regulatory expectations, and the growing pressure to balance rapid market access with rigorous compliance.

ATMPs are subject to stringent and continuously evolving regulatory frameworks, such as the European Medicines Agency (EMA) ATMP guidelines and the US FDA regenerative medicine framework. Consequently, QPs must now navigate not only GMP specific to ATMPs, but also issues related to tissue and cell sourcing, gene editing, and viral vector safety. In practice, this means ensuring compliance with both pharmaceutical and biotechnology standards.

The increasing adoption of digital technologies, including electronic batch records, laboratory information management systems (LIMS), and blockchain for traceability, has added further complexity. QPs are responsible for ensuring that these systems are validated, secure, and auditable. Similarly, automation and closed-system robotics offer opportunities to reduce human error; however, they also require QPs to validate new technologies and confirm process robustness, which is particularly critical in CGT, where the process itself defines the product.

Given the rapid pace of innovation, QPs must remain continuously informed about new regulatory guidelines, emerging technologies, and evolving case studies to effectively safeguard both compliance and product quality.

Q Further to this, what are the biggest challenges QPs currently face when overseeing batch release and ATMP production, and how can this be addressed?

HN QPs encounter several unique challenges when overseeing the batch release of ATMPs. One of the most critical issues is managing out-of-specification results when ensuring compliance with evolving GMP guidance. Unlike traditional medicines, ATMPs may, under certain circumstances, be released even if they do not fully meet specifications. This is only provided there is a document request from the treating physician and appropriate risk assessment, and mitigation measures are in place prior to final release to the patient.

Batch-to-donor variability presents additional complexity. Variability in donor-derived starting material can significantly influence the final product's quality and characteristics. This requires product specifications that are both robust and adaptable. The validation of analytical methods for ATMPs is also particularly challenging due to the heterogeneity of starting materials, making it difficult to standardize QC procedures fully and consistently demonstrate method reliability across batches.

Another key challenge is that some ATMPs are released as fresh products, without cryopreservation. These therapies must be administered to patients shortly after production, which imposes strict stability and shelf-life limitations. In such cases, QC testing must be accelerated, and release decisions may need to be made before all final test results are available. This necessitates the use of conditional release strategies, typically based on a risk-based approach to balance the urgency of treatment with the assurance of product quality and patient safety.

"For manufacturing teams, real-time communications with QA are critical to immediately address deviations and unexpected results."

Q In your work with early clinical phase and commercial products, what strategies have proven most effective for accelerating product release without compromising patient safety?

HN For advanced therapy developers, engaging with a CDMO at the earliest stage is essential. This ensures that QC strategies, analytical methods, and regulatory expectations are aligned well before clinical scale-up. Early dialogue supports an established QC framework that is fully compliant with regulatory standards and reduces delays later in development.

Accelerating the release of ATMPs while safeguarding patient safety remains a critical challenge, but several strategies have proven effective. The adoption of digital tools, such as manufacturing execution systems (MES) and LIMS, enables real-time monitoring, ensures data integrity, and supports faster batch release decisions.

Another important area is the early development and validation of analytical methods, particularly potency assays. Establishing potency assays early in the process prevents bottlenecks during clinical and commercial phases. Increasingly, multiplex analytical methods are being implemented to shorten testing timelines and reduce sample volumes. For example, the Bio-Techne Ella platform offers a high-throughput alternative to traditional ELISA, while the Charles River Endosafe accelerates endotoxin testing. These innovations contribute significantly to reducing the overall timeframe required before drug product release, without compromising safety or quality.

Q Can you share how cross-function collaboration between manufacturing, QA, and regulatory teams supports rapid yet compliant product release?

HN Cross-functional collaboration between manufacturing, QA, and regulatory teams is essential to achieving both speed and compliance in the release of ATMPs. Each function contributes distinct yet complementary expertise that, when integrated, accelerates release timelines without compromising quality or regulatory adherence.

For manufacturing teams, real-time communications with QA are critical to immediately address deviations and unexpected results. Close collaboration with regulatory teams ensures that process optimization or changes are implemented without compromising compliance.

For QA teams, early involvement in manufacturing planning helps align quality requirements and testing strategies from the outset. Rapid review of batch records and test data, combined with timely feedback to manufacturing, enables swift resolution of issues and deviations. In addition, QA works with regulatory colleagues to conduct risk assessments that prioritize critical attributes and mitigate potential compliance risks.

"...integration with enterprise resource planning and process control systems facilitates seamless information flow and supports compliance..."

For regulatory teams, proactive engagement with both manufacturing and QA is key. This includes interpreting evolving regulatory expectations, guiding process design, and streamlining documentation to align manufacturing outputs with regulatory submission requirements. Regulatory teams also play a central role in establishing and supporting change control processes to ensure that any updates to the manufacturing or quality system remain compliant.

Collectively, this triad of collaboration fosters an integrated approach that enables timely product release while safeguarding patient safety and regulatory compliance.

Q What innovations or tools have you seen reduce turnaround times for ATMPs while also ensuring robust safety data?

HN One of the most impactful innovations has been the implementation of MES solutions specifically designed to track and document the transformation of raw materials into finished drug products in real time. For ATMPs, MES platforms provide end-to-end visibility and traceability of manufacturing processes, which is essential for ensuring both quality and safety. Automated data capture of critical quality attributes and process parameters reduces manual error and accelerates quality assurance review for batch release. Furthermore, integration with enterprise resource planning and process control systems facilitates seamless information flow and supports compliance with regulatory requirements.

At CELL*for*CURE by Seqens, we have chosen to implement Körber PAS-X MES 3.3 as a cornerstone of our digitalization strategy for manufacturing traceability and documentation. PAS-X MES is being deployed as a standard, out-of-the-box SaaS solution. Modern cloud-based MES platforms offer scalability, flexibility, and improved access to data across multiple sites, which is an especially valuable feature for ATMPs, given their complex, patient-specific workflows.

Another important innovation at CELL*for*CURE has been the establishment of internal QC laboratories. By internalizing >90% of QC activities, we can maximize responsiveness, ensure robust tracability of results, and strengthen the verification of safety, identity, and potency. This organizational model supports both rapid turnaround times and the generation of reliable safety data, which are critical requirements for ATMPs.

Q As manufacturing scales up, how can environmental monitoring systems be optimized to ensure ongoing compliance without becoming a bottleneck?

HN At CELL*for*CURE, we have implemented the Growth Direct® System from Rapid Micro Biosystems to manage environmental monitoring (EM)

samples from our GMP facility, including in-process monitoring. This system, based on non-destructive microbial detection technology, enables fully automated incubation, detection, and enumeration of samples, significantly streamlining EM workflows.

The Growth Direct System detects the cellular autofluorescence of growing microcolonies. When illuminated with blue light, cells fluoresce in the yellow-green range, with oxidized flavin acting as a key fluorophore. This system distinguishes microbial colonies from non-biological fluorescent particles by superimposing multiple sequential images and subtracting static fluorescence signals that do not increase in size. Because the blue-light illumination is non-destructive, colonies can subsequently be identified using standard microbiological techniques.

Internal validation studies at CELL*for*CURE demonstrated that a final readout can be obtained after 56 hours of incubation, compared with a minimum of 120 hours using conventional incubation methods. This reduction in time has drastically accelerated EM sample management, preventing monitoring from becoming a bottleneck while ensuring compliance and maintaining high microbiological quality standards.

Q Looking ahead, what do you think will be the most critical changes or innovations needed in CGT manufacturing to ensure faster, safer, and more scalable delivery of advanced therapies to patients?

HN CGT manufacturing is at a pivotal stage, with the potential to transform medicine, but still facing major challenges in scalability, cost, and speed. Several critical innovations will be necessary to accelerate delivery while maintaining safety and quality.

First, automation and closed systems will be fundamental. Fully automated, closed platforms for cell culture, gene editing, and cryopreservation can reduce contamination risk, improve reproducibility, and accelerate production timelines. Advances in QC technologies, particularly miniaturized and rapid-testing platforms, are also becoming increasingly important. Tools such as Ella, BioFire Mycoplasma, and Endosafe illustrate how innovative technologies can shorten release timelines while reducing manual steps, reagent consumption, and associated costs.

Second, the standardization of protocols will be crucial. Standardized methods for different cell therapy types, such as CAR-T therapies using lentiviral vectors, would facilitate faster deployment and simplify technology transfer between facilities. Initiatives such as T2EVOLVE, part of the European Union Innovative Medicine Initiative (IMI), are already advancing the standardization and acceleration of development, manufacturing, and QC for CAR-T cell therapies.

Third, regulatory evolution will play a defining role. In May 2025, the EMA released a concept paper proposing revisions to Part IV of the EudraLex Volume 4 guidelines on GMP for ATMPs. The aim is to align ATMP-specific GMP with the updated Annex I and to integrate recent advances in manufacturing technologies and quality management systems. Such regulatory updates are essential for Europe to remain competitive in the global ATMP landscape.

Finally, scale-up and product comparability remain central challenges. As ATMP manufacturing expands, ensuring product comparability after process changes is particularly complex. Regulatory authorities in the US (FDA), Europe (EMA), and Japan (MHLW) have

each issued guidance addressing this issue. Sponsors are strongly advised to engage proactively with regulators, especially when introducing high-risk process changes, to avoid clinical holds or delays in approval due to failed comparability assessments.

Overall, the integration of automation, standardized protocols, evolving regulatory frameworks, and robust scale-up strategies will be critical to ensuring that advanced therapies are delivered to patients more rapidly, safely, and at greater scale.

BIOGRAPHY

Hélène Negre has developed her expertise in cell and gene therapy bioproduction with over 20 years of experience in ATMPs. Initially, she worked at Pitié-Salpêtrière Hospital in the Biotherapy department. In 2014, she joined Dana Farber Cancer Institute, Boston, and worked 5 years as Technical Director of the Novel Cell Therapy team in the Cell Manipulation Core Facility with Professor Ritz. From 2019 to 2023, she worked at Servier as Scientific Director—Biotechnology and Bioproduction and EFPIA project leader for the IMI program T2Evolve on engineered T cells. In 2024, she joined CELL*for*CURE by Seqens as Pharmaceutical Affairs Director.

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AUTHORSHIP & CONFLICT OF INTEREST

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How do I get started with analytical assays?

CELL & GENE THERAPY INSIGHTS

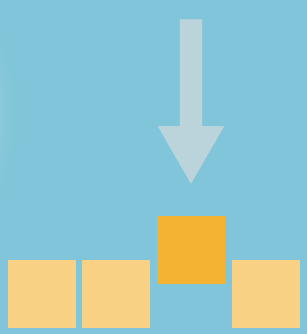
TOP TIPS

This is a common question for those faced with the complex and challenging task of manufacturing biotherapies in general, and cell therapies in particular. Here are four important points for consideration when deciding which assays are best suited to your individual product.

1 Regulatory guidelines and compliance

Firstly, it is crucial to select assays that are aligned with regulatory guidelines and pharmacopeial standards. The next step is to validate the analytical methods to demonstrate they are fit for the intended use and specific to your therapy.

2 Integration



How well can the sample-to-answer solution be integrated into your manufacturing and analytical processes? Choose assays with seamless workflows from sample through to result. Integrated platforms are preferable as they help minimize variability, streamline data management, and ensure reproducibility.

3 Ease of implementation and routine use

Seek to prioritize user-friendly, automated assays that are compatible with lab infrastructure in order to reduce both errors and training requirements.

4 Scalability

Last but certainly not least is the crucial aspect of scalability. Select high-throughput, flexible assays that are capable of supporting production from R&D through to commercial scale.

For more insights, watch this **On Demand webinar** on the **Analytics Hub**—your resource for practical advice and technological updates relating to analytical development for advanced therapies.