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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

Expanding the range of cellular immunotherapy

GUEST EDITOR

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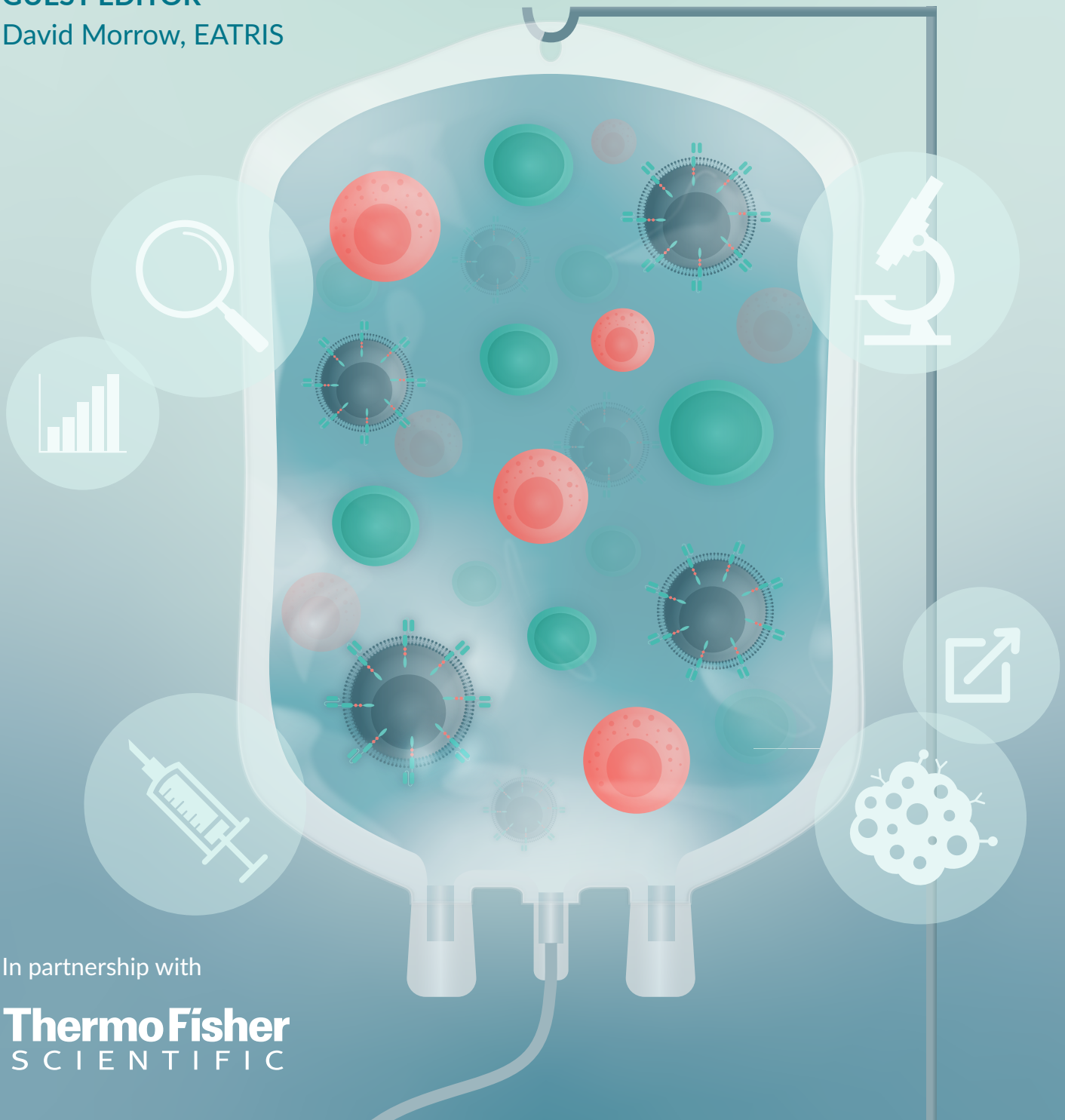

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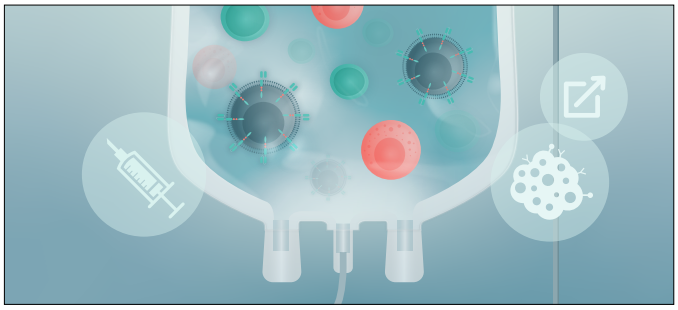

CELL & GENE THERAPY INSIGHTS

EXPANDING CELLULAR IMMUNOTHERAPY

SPOTLIGHT

Breaking barriers in cell and gene therapy: advancing ATMPs through innovation and collaboration

Anton Ussi, Toni Andreu, Florence Bietrix, and David Morrow

FOREWORD

"As a community, the ATMP field needs to build a new collaborative ecosystem that leverages data to tackle complex diseases, particularly rare genetic disorders."

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In recent years, a slew of advanced therapy medicinal products (ATMPs), particularly cell and gene therapies, have demonstrated the transformative power of these therapies. CAR-T cell therapy, for example, has revolutionized the field in the treatment of patients with various types of relapsed/refractory (R/R) B-cell malignancies including diffuse large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (B-ALL), follicular lymphoma (FL), mantle cell lymphoma (MCL), and multiple myeloma (MM). CAR-T cell application in solid tumors, however, remains elusive due to a variety of challenges in the biology. This notwithstanding, results published in 2024 brought renewed optimism for the use of CAR-Ts in solid tumors, by showing that targeting two brain tumor-associated

proteins instead of one showed promise as a strategy for reducing tumor growth in patients with recurrent glioblastoma (GBM), an aggressive form of brain cancer. According to results obtained by researchers from the Perelman School of Medicine at the University of Pennsylvania and Penn Medicine's Abramson Cancer Centre in an **ongoing Phase 1 clinical trial**, this 'dual-target' approach is showing early promise that CAR-T therapies for solid tumors like GBM can potentially generate effective enduring responses. It is clear, however, that to achieve the full potential of cell and gene therapies, it is crucial to continue to strive to overcome significant barriers in the biology, clinical application and regulatory science. Examples of current obstacles include limited understanding of cell behavior in patients, complicated regulations due to the rapid pace of technological changes, lack of data standardization, poor reproducibility in early development, and inadequate communication among stakeholders.

Central to advancing ATMPs such as CAR-Ts is addressing the obvious challenge in predicting how patients will respond. There is a strong need for specific biomarker signatures to help doctors locate the best candidates for these treatments. Technologies that observe immune cell activity are vital for assessing the effectiveness and duration of ATMP responses. New methods like non-invasive imaging and computer modelling are being explored for predicting treatment success and minimizing invasive testing. Such innovations could streamline patient selection in ATMP supply chains.

Developing safe and effective cell and gene therapies needs innovative technologies and collaborative approaches to address the technical challenges in research, development, and production. Platforms that adapt to specific needs using standardized tools are becoming increasingly popular for developing treatments and diagnostics, making the process more efficient and cost-effective. In 2021, the National Institute of Health (NIH), the US FDA, and various organizations started the Bespoke Gene Therapy Consortium (BGTC) to create standards that speed up the development of personalized gene therapies. Further similar initiatives are now essential, particularly in the CAR-T space as we strive to create more effective, safer, and cost-effective development processes for streamlining their development. To achieve this goal, the right collaborations and incentives must be fostered and facilitated where industry and academia—together with patient organizations—can work together to develop such standardized platform technologies. One recently launched example of such an initiative is the **European Rare Diseases Research Alliance (ERDERA)**, which launched in September 2024. ERDERA aims to improve the health and well-being of the 30 million people living with a rare disease (RD) in Europe, by making Europe a world leader in RD research and innovation and support concrete health benefits to patients, through better prevention, diagnosis, and treatment. This partnership aims to deliver an RD ecosystem that builds on the successes of previous programs by supporting robust patient need-led research, developing new diagnostic methods and pathways, and spearheading the digital transformational change connecting the dots between care, patient data, and research, while ensuring strong alignment of strategies in RD research across countries and regions. A core component of ERDERA is to develop innovative platform technologies that facilitate viral and non-viral gene therapy development. This represents a first of its kind initiative in the EU where industry and public partners work together to develop such platform technologies with the goal to facilitate a more standardized and streamlined development process for

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gene therapies for RD in a manner similar to the successful Bespoke Gene Therapy Consortium (BGTC) in the USA.

Europe needs to effectively facilitate collaboration among research institutions, patients, healthcare professionals, industry, and regulators to fully utilize resources for developing safer and more effective ATMPs. One of the ERDERA partners, the European Research Infrastructure for Translational Medicine (EATRIS), is working to support ATMP development by providing the right innovative tools and technologies to researchers to support their ATMP development programs. This is achieved by an infrastructure of over 150 research institutions and medical centers across 14 EU countries with an ambition to support translation of innovative therapies such as ATMPs to the clinic. To achieve this, EATRIS strives in particular to connect regional ATMP infrastructures and communities to establish a strong ecosystem for ATMP creation in Europe, benefiting academia and industry alike. By creating a robust ecosystem with reproducibility and quality at its core, effective ATMPs can meet their potential and revolutionize treatments for RD, cancer, auto-immune, and other pathologies in the future.

Research continues apace on strategies to avoid or dampen adverse effects from ATMPs, including managing cytokine release syndrome (CRS), a serious complication from some treatments. This could potentially be achieved through standardized technology platforms, as mentioned above, that address for example therapy response in the individual. New and effective prognostic tools can help doctors manage CRS early for example, balancing risks and treatment efficacy. As a community, the ATMP field needs to build a new collaborative ecosystem that leverages data to tackle complex diseases, particularly rare genetic disorders. While the concept of ATMPs might seem straightforward, actual implementation is complex and needs a profound understanding of personalized treatment strategies. Addressing these complexities through targeted research and innovation should remain a priority as we seek to enhance ATMP efficacy and improve patient outcomes.

BIOGRAPHIES

Toni Andreu specialized in genetics and genomics of rare diseases. He has been working in the field of neuromuscular disorders from a clear translational perspective, from basic science to the development of cell and animal models and clinical research. During his career he has published over 180 scientific papers, numerous book chapters and supervised several PhD programs. After working at Columbia University on mitochondrial disorders from 1998–2001, he moved to Barcelona to create the Neuromuscular Lab at the Vall d'Hebron Research Institute where he became Director of the Neurosciences Research Program and later CEO of the University Hospital of Bellvitge, one of the largest health care institutions in Spain. He has also been extremely active in the field of policy-making, and has held positions as the Director of the Spanish National Institute of Health Carlos III as well as the Director General for Research and Innovation at the Catalan Ministry of Health. Toni is the current Scientific Director of The European Infrastructure for Translational Medicine (EATRIS), Amsterdam, The Netherlands a position he has held since 2018.

Anton Ussi is Operations and Finance Director at EATRIS ERIC, the ESFRI European infrastructure for translational medicine. Joining EATRIS in 2010, he was part of the team responsible for the design and statutory incorporation of the infrastructure, whose membership

counts 14 European member states. In his current role as CEO since 2015, Ussi has a background in technology transfer, with previous history in mechanical engineering and automotive design, and small business administration. He specializes in public–private and public–public collaboration and translational research in medicine. Ussi is also Principal Investigator of REMEDi4ALL, a large EU-funded initiative to develop a European medicines repurposing framework.

Florence Bietrix is the Deputy Director to the Executive Board at EATRIS. She earned her PhD in Life Sciences from Toulouse University, Toulouse, France in 2006. Following her doctorate, she worked as a study director at Avogadro, a contract research organization in Fontenilles, France, where she managed pre-clinical studies on new therapeutics for atherosclerosis and metabolic syndrome. In 2008, Bietrix relocated to the Netherlands to undertake postdoctoral research at the Academic Medical Center in Amsterdam. Her research focused on developing new therapeutic targets for atherosclerosis and nonalcoholic steatohepatitis. She joined EATRIS as a Scientific Platform Manager for Biomarkers and Advanced Therapy Medicinal Products (ATMPs) and gradually transitioned into operations. Since 2019, she has served as the Head of Operations, overseeing strategic development, quality assurance, and implementation of initiatives within the organization. Her dedication to translational research reflects a holistic approach, from preclinical study design to fostering international collaboration, ensuring scientific discoveries lead to tangible benefits for patients.

David Morrow is the ATMP and Vaccine Scientific Program Manager at EATRIS C&S. David received a BSc (Hons) in Molecular Biology from University College Dublin, Dublin, Ireland in 2001 and a PhD in Vascular Biology from Dublin City University, Dublin, Ireland in 2006. This was followed by an American Heart Association Postdoctoral Fellowship at the University of Rochester Medical Center, NY, USA from 2006–2008 which resulted in an American Heart Association Young Investigator Award in 2008. From 2009–2015 he was an NIH/American Heart funded Principal Investigator heading multiple projects in cardiovascular disease and cancer. David also holds an MBA in Health Science Management and has worked as a consultant and in technology transfer in life sciences. David has recently authored papers and being invited as guest speaker at panel discussions at international conferences discussing the major bottlenecks in ATMP development.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

EXPANDING CELLULAR IMMUNOTHERAPY

SPOTLIGHT

COMMENTARY

CAR-T cells in B-cell mediated autoimmune diseases: do all roads lead to Rome?

Dimitrios Mougiakakos

The use of CAR-T cell therapies in autoimmune diseases represents a groundbreaking shift in treatment paradigms. Building on successes in hematologic oncology, CAR-T cells targeting CD19 have shown profound efficacy in achieving durable remissions, as exemplified by the first patient treated for severe systemic lupus erythematosus. These therapies offer advantages such as active migration of a 'living' drug, deep tissue penetration, and potential immunologic reset over traditional B-cell depleting strategies. Complementary approaches, including bispecific antibodies and emerging cell-based modalities such as allogeneic CAR-T cells or CAR NK cells, are expanding the therapeutic armamentarium. While promising, challenges remain with respect to scalability, accessibility, and long-term outcomes. Ongoing research heralds a paradigm shift in personalized, durable autoimmune therapies.

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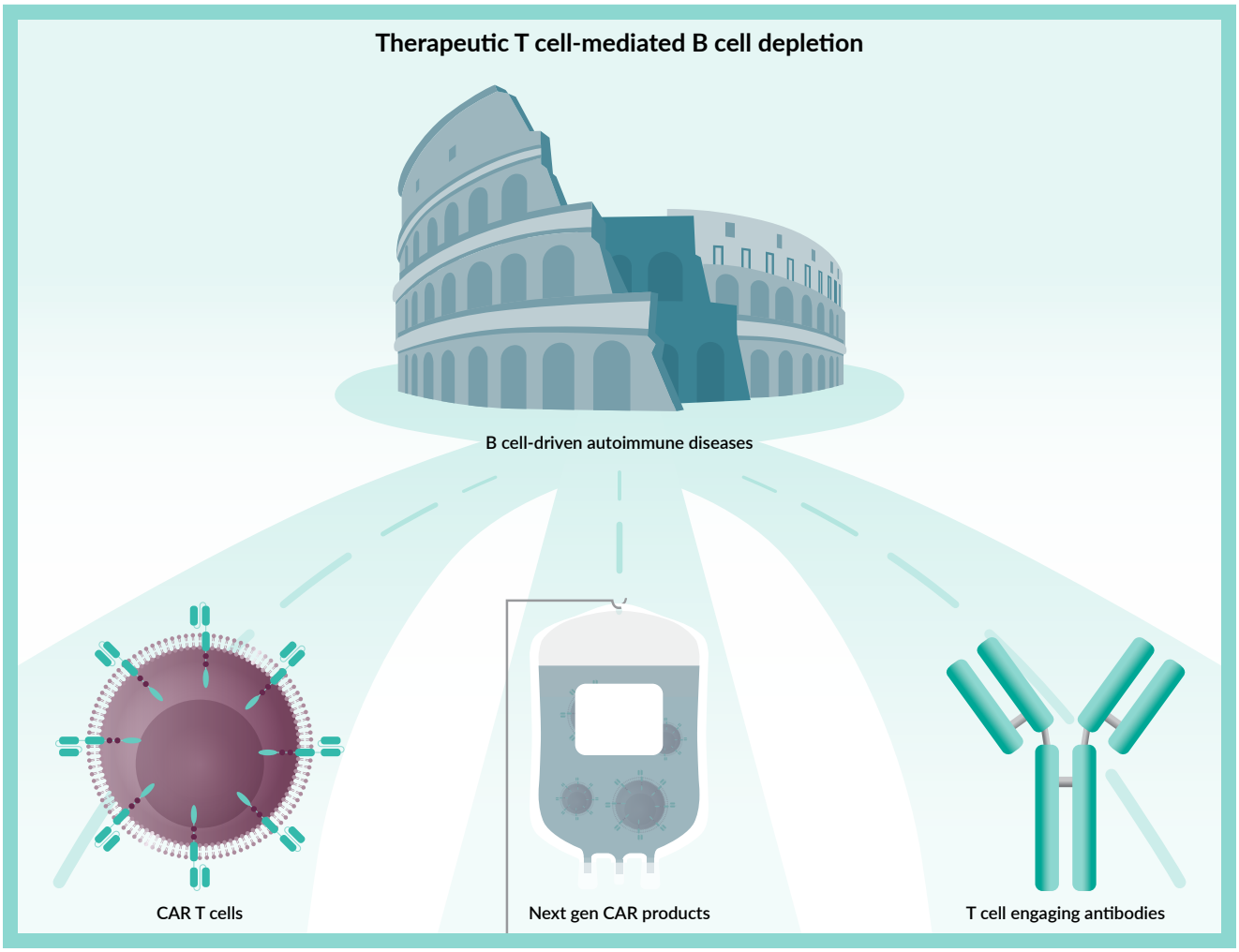
Not much longer than 3.5 years ago in 2021, our team faced significant challenges in treating a young patient with severe systemic lupus erythematosus (SLE) [1]. Multiple prior therapeutic interventions, including B-cell-depleting monoclonal antibodies, had failed to achieve sustained disease stabilization. In response, we, together with the patient, elected to pursue a novel approach: the administration of CAR-T cells targeting the CD19 antigen on B cells.

RATIONALE FOR CAR-T CELLS AND CD19 TARGETING

B cells are integral to the pathophysiology of numerous autoimmune diseases, including SLE [2]. Their pathogenic roles extend beyond autoantibody production, such as anti-double-stranded DNA (anti-dsDNA) antibodies in SLE, to include antigen presentation (of self-peptides) and the secretion of pro-inflammatory cytokines like interleukin (IL)-6. In a similar manner, multiple sclerosis



Road(s) to Rome: T cell-mediated B-cell depletion as a promising therapeutic approach for B-cell-driven autoimmune diseases. Currently, different strategies are being explored for clinical implementation: the use of established CAR-T cells, well known from real-world experience in hematology and oncology; novel CAR technologies originally developed for hematologic malignancies, now being directly evaluated in autoimmune diseases; and T cell engagers, which enable targeted immune modulation without the need for cell transfer. All these approaches share the common goal of effectively addressing the challenges in the treatment of autoimmune diseases.

(MS) is characterized by the presence of intrathecal oligoclonal bands (OCBs), which originate from clonally expanded B cells within the brain parenchyma that produce immunoglobulins. Likewise, in rheumatoid arthritis (RA), hallmark autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) play a key pathogenic role. Consequently, B-cell depletion strategies have been employed in various autoimmune conditions; for instance, the monoclonal anti-CD20 antibody rituximab is approved for RA [3] or is regularly used off-label in MS [4].

While CAR-T cell therapies have primarily been approved for hematologic malignancies, such as lymphomas and multiple myeloma, their profound and sustained B-cell depletion capabilities suggest potential applicability in B-cell-driven autoimmune diseases [5,6]. Notably, CAR-T cells exhibit active migration, longevity, and effective tissue penetration, potentially surpassing the depletion achieved by monoclonal antibodies, particularly within tissue compartments. Unlike antibodies, which rely solely on passive diffusion and have a limited half-life, CAR-T cells

actively home to target sites and persist longer *in vivo* [7]. This distinction is particularly relevant in diseases such as SLE, where we have observed that successful B-cell depletion in peripheral blood did not necessarily correlate with effective elimination in affected tissues, such as the renal parenchyma [8]. Recent studies have demonstrated deeper B-cell depletion in lymph nodes with CAR-T cells compared to monoclonal antibody treatments [9].

Targeting CD19 offers the advantage of broad expression across various B-cell differentiation stages, including plasmablasts and subsets of plasma cells [10]. This broad targeting is advantageous for addressing the diverse pathogenic B-cell functions in autoimmune diseases. On the other hand, it cannot be excluded that in certain disease entities or even on a personalized level, it may be more beneficial to also target CD19-negative, CD38- or BCMA-positive plasma cells [11,12]. To determine the optimal approach, further data are needed, ideally including the identification of predictive biomarkers. Additionally, a thorough risk–benefit assessment is required, particularly regarding the potential consequences of the resulting immunodeficiency.

CLINICAL OUTCOME AND IMMUNOLOGICAL RESET

When deciding on the treatment, one concern was the potential exacerbation of inflammation by infusing CAR-T cells into a highly inflammatory environment or the at least theoretical risk of transducing an autoreactive T cell clone. However, the patient tolerated the infusion well and showed a rapid reduction in symptoms, achieving and maintaining treatment free remission to date [13]. This finding suggests an 'immunological reset' as B cells reconstituted as predominantly naive cells without autoantibody production. In addition to the 'rejuvenation' of the B-cell population, changes in the gene expression signatures of other immune cells such as monocytes and T cells have also been observed, indicating for example reduced inflammatory activity, such as an attenuated IFN signature [14]. Overall, this process is still insufficiently understood and requires further research.

BROADER IMPLICATIONS AND ONGOING RESEARCH

This successful outcome has generated significant interest in both academic and industrial sectors, with the aim of achieving long-lasting, potentially permanent, therapy-free remissions, ideally with a single infusion of CAR-T cells [6,7]. Given the increasing incidence of autoimmune diseases, this approach holds great promise, although questions remain about future accessibility and scalability.

In the initial phase of exploration, established CAR-T cell therapies from hematology and oncology have been applied to a growing number of therapy-refractory patients and autoimmune indications with underlying B-cell pathophysiology, including SLE, systemic sclerosis (SSc), and myositis [13,15,16]. These studies have employed autologous CAR-T cells of the second generation with 4-1BB or CD28 co-stimulatory domains targeting antigens like CD19 or B-cell maturation antigen (BCMA), administered following lymphodepletion with agents such as fludarabine and cyclophosphamide [6].

Emerging data from these studies have confirmed the anticipated therapeutic potential, with some reports indicating remarkable efficacy. Notably, the safety profile in autoimmune patients appears more favorable compared to oncology settings, with lower incidences and severities of cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), hematotoxicity, and infections [5,6]. In a recently published meta-analysis of a total of 80 patients

with autoimmune disorders (i.e., SLE, SSc, idiopathic inflammatory myositis, antiphospholipid syndrome, RA, and Sjögren's syndrome) treated with B-cell-depleting CAR-T cells, it was found that all patients achieved an immunosuppression-free state at the last follow-up [17]. Regarding tolerability, grade ≥2 CRS was rare, occurring in only four cases, and ICANS was also reported in four cases, both of which were well manageable. No fatal adverse events occurred. This disparity underscores the distinct differences between patient populations and cautions against direct extrapolation from oncology to autoimmune contexts. However, it is important to note that these results are based on a limited number of studies and patients. Larger, randomized studies are needed to determine more precise efficacy rates and the incidence of adverse events such as CRS and ICANS.

The observed favorable safety profile may be attributed to factors such as differing pre-treatments (i.e., less myelotoxic), biological reserves, and notably, the lower antigen burden in non-malignant diseases. Nonetheless, isolated reports of severe adverse events in clinical studies warrant continued vigilance, especially as patient numbers increase and interindividual variability becomes more apparent.

To date, the scope of CAR-T cell therapy has expanded beyond rheumatology to include B-cell driven neuroimmunological diseases. B-cell-depleting CAR-T cells have been utilized in conditions affecting both the peripheral (e.g., myasthenia gravis [18] or stiff person syndrome [19]) and central nervous systems (CNS; e.g., MS), yielding promising results in terms of efficacy and tolerability and numerous early clinical trials are being planned or are already recruiting patients. Here, too, the paradigms from hemato-oncology need to be reconsidered, as we may have to monitor and evaluate patients with CNS involvement in a completely different way with regard to toxicities, especially ICANS (e.g., by using continuous EEG) [20].

ADVANCEMENTS IN CAR-T CELL TECHNOLOGY

Before achieving regulatory approval for CAR-T cells in autoimmune diseases, a second phase of development has commenced, leveraging nearly a decade of experience with CAR-based technologies in malignancies. This progression allows the field of autoimmune diseases to potentially bypass certain developmental stages through a 'dual track' approach.

Innovations under investigation include multispecific CAR-T cells capable of recognizing multiple antigens to mitigate the risk of immune escape. One example is BCMA-CD19 compound CAR-T cells, which are being evaluated in SLE patients [21]. In addition, mRNA-based CAR-T cells have been explored, offering advantages such as reduced risk of insertional mutagenesis and transient CAR expression, providing an additional safety mechanism in cases of CAR-T cell-induced toxicity but requiring repeated dosing. Notably, mRNA-based approaches may eliminate the need for lymphodepletion, leading to entirely 'chemotherapy-free' treatments. However, comparative efficacy between transiently and stably expressed CARs remains to be fully elucidated. Here, too, the first promising data from clinical trials with BCMA CAR-T cells in MG are already available [22].

CAR natural killer (NK) cells are emerging as a promising alternative to CAR-T cells in autoimmune disease therapy, leveraging their distinct biological properties and favorable safety profile [23]. Unlike T cells, NK cells belong to the innate immune system, offering both CAR-mediated and natural cytotoxicity, which enables them to target autoreactive immune cells effectively. CAR NK cells have shown reduced risks of severe CRS

and ICANS, as they release fewer pro-inflammatory cytokines such as IL-6. Their limited lifespan *in vivo* also mitigates long-term toxicity, providing a built-in safety mechanism. These cells are particularly well-suited for allogeneic 'off-the-shelf' therapies, as they are less prone to causing graft-versus-host disease (GvHD) and can be engineered from healthy donors or induced pluripotent stem cells (iPSCs). Challenges such as limited persistence and scalability remain, but advancements like IL-15 overexpression hold promise.

In the realm of T cell effectors, allogeneic CAR-T cells are garnering significant attention, offering the potential for readily available 'off-the-shelf' treatments [24]. Unlike autologous CAR-T cells, which require patient-specific manufacturing, allogeneic CAR-T cells are derived from healthy donors, enabling large-scale production and rapid accessibility. This approach addresses critical limitations of autologous therapies, such as long manufacturing times, complex logistics including apheresis, high costs (of approximately US$350,000–450,000), and dependence on the patient's immune cell health, which may be compromised in autoimmune diseases. Lymphodepletion may always be necessary in the allogeneic approach to ensure the persistence of the transferred cells. To overcome risks like GvHD and early rejection, allogeneic CAR-T cells are engineered with sophisticated genetic modifications. Techniques such as CRISPR/Cas9 are used to knock out the T cell receptor (TCR) to prevent GvHD and modify HLA molecules to evade immune rejection. Early clinical reports have shown that these cells can effectively target pathogenic immune populations in autoimmune diseases like SSc and myositis while maintaining safety [25,26]. The possible reduction in costs could ultimately be the decisive aspect in enabling better access to healthcare systems that are under great strain worldwide.

Further potential effector populations include γδ T cells [27] as well as professional immunoregulatory cell populations such as double-negative (DN) T cells [28], regulatory T cells (TRegs) [29], and mesenchymal stromal cells (MSCs) [30]. They are designed to recognize disease-specific antigens, dampen immune activity through cytokine release and modulation of antigen-presenting cells, and induce bystander suppression in affected tissues, which means that the target cell (i.e., B cell) is not directly destroyed and could have certain advantages in terms of preserving immune function. These cell-based therapies, currently being primarily evaluated in preclinical settings, hold significant potential, and we eagerly await the first patient data to understand their clinical impact and scalability [7].

ALTERNATIVE APPROACHES TO T CELL-MEDIATED B-CELL ELIMINATION IN AUTOIMMUNE DISEASES

In parallel with the development and clinical evaluation of CAR-T cell therapies for autoimmune diseases, the concept of T cell-mediated B-cell elimination is being explored as a complementary or competitive strategy. These efforts build on the extensive experience in hemato-oncology, where bispecific antibodies have significantly changed treatment paradigms. Bispecific antibodies, such as those targeting CD3 and CD19 (e.g., blinatumomab) or BCMA (e.g., teclistamab), establish a 'rendezvous' between the effector T cell and the target cell (e.g., CD19pos B-cells or BCMApos plasma cells). This interaction activates the T cell and triggers the destruction of the target cell in a repeatable cascade.

In oncology, the introduction of bispecific antibodies has reshaped the treatment landscape, raising recurring questions about the optimal selection and

sequencing of bispecific antibodies versus CAR-T cells [31]. While the curative potential of CAR-T cells is well established, bispecific antibodies lack sufficient long-term follow-up to confirm such results. Their advantages include off-the-shelf availability, potentially better tolerability, and lower cost for short-term use if they could replicate the efficacy of CAR-T cells in certain contexts of autoimmunity. Moreover, due to their more favorable toxicity profile, they could also be administered to patients who are not suitable for CAR-T cell therapy due to their overall condition. However, questions remain about the depth of depletion achievable with bispecific antibodies, particularly given their lack of active tissue penetration comparable to CAR-T cells.

Not surprisingly, following the early successes of CAR-T cell therapy, the first patients with refractory autoimmune diseases across the rheumatologic spectrum were treated with bispecific antibodies in CD3×CD19 and CD3×BCMA formats [32–34]. The results, although based on limited follow-up, were very promising and generated considerable enthusiasm, leading to numerous active clinical trials. Thus, the field now encompasses not only two parallel tracks involving CAR-T cell therapies, but also a third track addressing T cell-based strategies broadly in the context of B-cell-mediated autoimmune diseases.

OUTLOOK

The rapid development of CAR-T cell therapies and complementary strategies, such as bispecific antibodies and novel effector cell populations, underscores a transformative era in the treatment of autoimmune diseases. While early successes, such as durable remissions in SLE, highlight the potential of these innovative therapies, many questions remain regarding long-term outcomes, accessibility, and scalability. The introduction of 'off-the-shelf' solutions, such as allogeneic CAR-T cells and bispecific antibodies, offers promising ways to overcome logistical and cost barriers. In addition, emerging technologies, including mRNA-based CAR-T cells and CAR NK cells, herald a future in which therapies are safer, more versatile and more widely available. As preclinical research advances and more clinical trials report data, the field is approaching a paradigm shift that offers hope for durable, targeted, and personalized treatments. The bench-to-bedside journey continues to hold immense potential, and the collaborative efforts of academia and industry are poised to redefine therapeutic standards of care in autoimmune disease.

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CELL & GENE THERAPY INSIGHTS

EXPANDING CELLULAR IMMUNOTHERAPY

SPOTLIGHT

INNOVATOR INSIGHT

Worth the switch? Enhancing process performance for cell therapy manufacturing with an animal component-free raw material strategy

Phil Morton and Shanya Jiang

Historically, cell therapies have relied heavily on animal- and human-derived components—but the variability associated with these sources can lead to inconsistency and safety concerns. In recent times, focus has shifted toward utilizing chemically-defined raw materials, including animal component-free proteins. This article explores the critical role raw materials play in process performance and highlights how animal component-free raw materials can enable optimized, reliable, and efficient cell therapy manufacturing.

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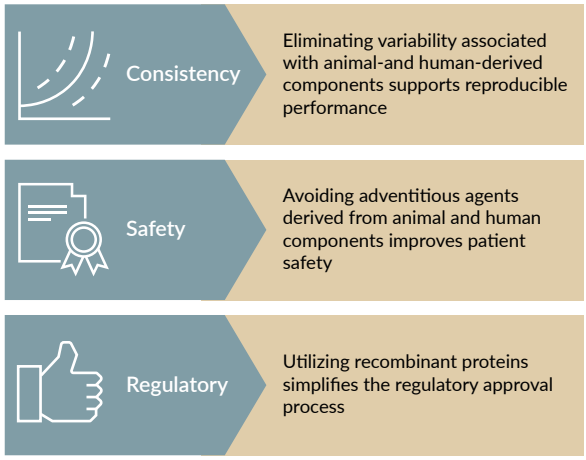
Throughout the cell therapy manufacturing workflow, various raw materials from cell culture media (including albumin) cytokines, biopreservation solutions, and stabilizing agents are utilized. While some materials, such as excipients, become part of the final formulation and others like ancillary materials do not, the quality of all materials that come into contact with cells is critically examined. This is because material quality can directly influence the stability, safety, potency, and purity of the final product. Shifting from the use of animal and human-derived raw materials to animal component-free raw materials in cell therapy manufacturing can address both safety concerns and consistency challenges (Figure 1). For example, human serum albumin often contains numerous ligands when derived from serum-based processes. These ligands can vary depending on the raw material source, geography, and supplier—and even from batch to batch, despite efforts by suppliers to minimize variability through pooled donations. By transitioning to a recombinant version, a more consistent process can be achieved. This approach also enhances safety and

FIGURE 1

Benefits of using aninal component-free raw materials in cell therapy manufacturing.



regulatory compliance, leading to faster regulatory approvals. In addition, improved performance and consistency can ultimately reduce overall cost per dose, offering a clear advantage for a manufacturing process.

Given these benefits, many raw material suppliers have expanded their portfolio to include chemically-defined components. Below we explore the advantages of an animal component-free raw material strategy, from recombinant human albumin, to cell culture media, cytokines, and cryopreservation solutions.

IMPROVED CRYOPRESERVATION AND POST-THAW RESULTS WITH RECOMBINANT HUMAN ALBUMIN

Recombinant human albumin is a critical material for many cell-based products and is widely used due to its versatility. As a carrier molecule, it can be used for cell banking, cell culture media, harvesting, formulation, and cryopreservation.

The molecule's core properties, outlined in Figure 2, provide a number of benefits. Sartorius's recombinant human albumin, Recombumin®, has supported customers in various applications from cell growth to cryopreservation. It is manufactured using a quality management system certified to ICH Q7 GMP guidelines.

In addition to to its physiochemical and biological benefits, Recombumin can be further customized to optimize its performance for different applications as it is fully recombinant, chemically defined, and ultra-pure. This allows the ligand and additive profiles to be altered for optimization to a specific process, for example, cell expansion as illustrated in Figure 3.

Building on its customizable nature, Recombumin also demonstrates significant benefits in cryopreservation, particularly for T cells. When compared to serum-derived albumin, Recombumin shows superior cryopreservation performance and improved cell recovery post-thaw due to its consistent and chemically defined composition (Figure 4).

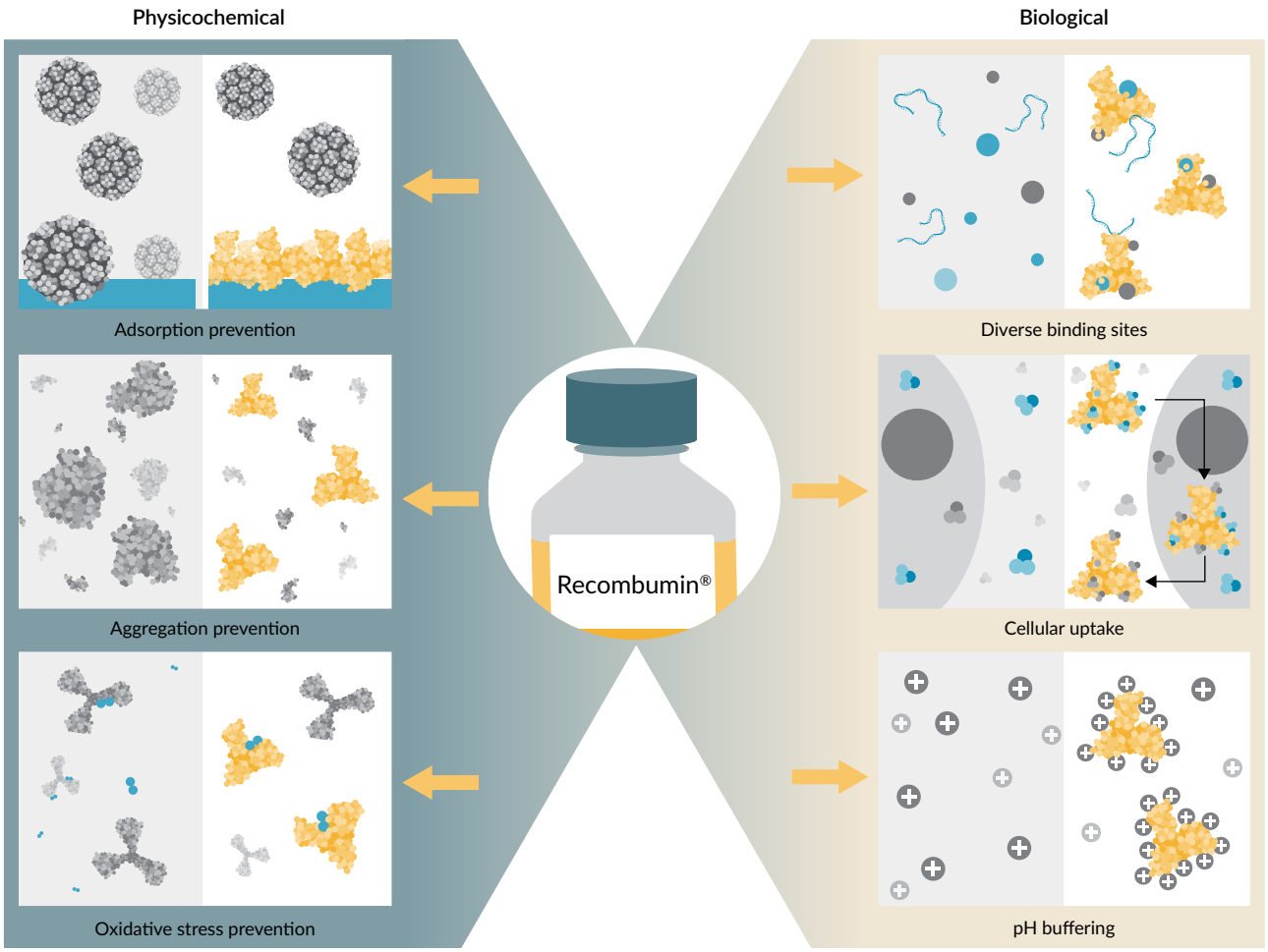
While serum-derived albumin may contain beneficial ligands, it also introduces potentially undesirable ligands that can adversely affect cells. Recombumin eliminates this variability, delivering a more reliable and effective solution.

Using Recombumin in cryopreservation also offers the possibility of reducing dimethyl sulfoxide (DMSO) content (Figure 5). There is a growing interest in reducing or eliminating DMSO from cell therapy formulations due to the negative side effects (for example, gastrointestinal, cardiovascular, respiratory, and dermatological reactions) that it can have on patients.

The advantages of recombinant human albumin extend beyond cryopreservation to the critical 2-hour window after thawing T cell therapies. As Figure 6 illustrates, Recombumin preserves a higher number of live cells while reducing the proportion of dead and apoptotic cells over the 2-hour period generally allowed for CAR-T cell therapies before administration with benefits extending up to 4 hours. This extended window provides clinicians with more time to administer these life-saving therapies without compromising their efficacy or quality.

FIGURE 2

Properties of recombinant human albumin, like Sartorius' Recombumin®.



Furthermore, Recombumin is supported by drug master files (DMFs) in the USA, Japan, Canada, New Zealand, China, and Australia. While Europe does not have such a system, there are mechanisms in place to address this. These DMFs, along with the regulatory support offered by Sartorius, provide a basis for safety in the process.

CHEMICALLY DEFINED T CELL MEDIA FOR INCREASED CELL EXPANSION AND VIABILITY

Cell culture media plays a pivotal role in providing a nutrient-rich environment for cells to grow and function properly. Traditionally, cell culture media has been supplemented with human serum as it provides the essential growth factors, hormones, and lipids for cell proliferation and growth. But concerns surrounding consistency, supply, and the risk of introducing adventitious agents have urged the field to transition towards serum-free and animal component-free alternatives. To address these concerns, Sartorius offers chemically defined T cell media, which has been developed to ensure robust performance, promote strong CAR-T cell growth, and maintain stable CAR expression without the addition of serum. This is achieved through the use of recombinant proteins, specifically Recombumin, to optimize the media formulation.

As shown in Figure 7, culturing peripheral blood mononuclear cells (PBMCs) from healthy donors with 4Cell® Nutri-T Advanced results in higher fold-expansion compared to alternative chemically defined media. It also provides comparable or better performance than media supplemented with or without serum, with viability of T cells being maintained above 90%.

Importantly, the use of 4Cell Nutri-T Advanced medium resulted in a 2.4-fold higher expansion of patient-derived CAR-T cells compared to alternative chemically defined media (Figure 8). Good viability of CAR-T cells was also maintained.

In terms of CAR expression, 4Cell Nutri-T Advanced media supports robust CAR expression over 10-day culture. The CD4/CD8 cell ratios are similar, maintaining balanced and functional cells (Figure 9).

From a functionality perspective, 4Cell Nutri-T Advanced medium produces highly cytotoxic cells, with even higher killing capacity at 1:4 effector:target ratio (Figure 10). This not only outperforms other chemically defined media but also shows slightly better results than media supplemented with human serum.

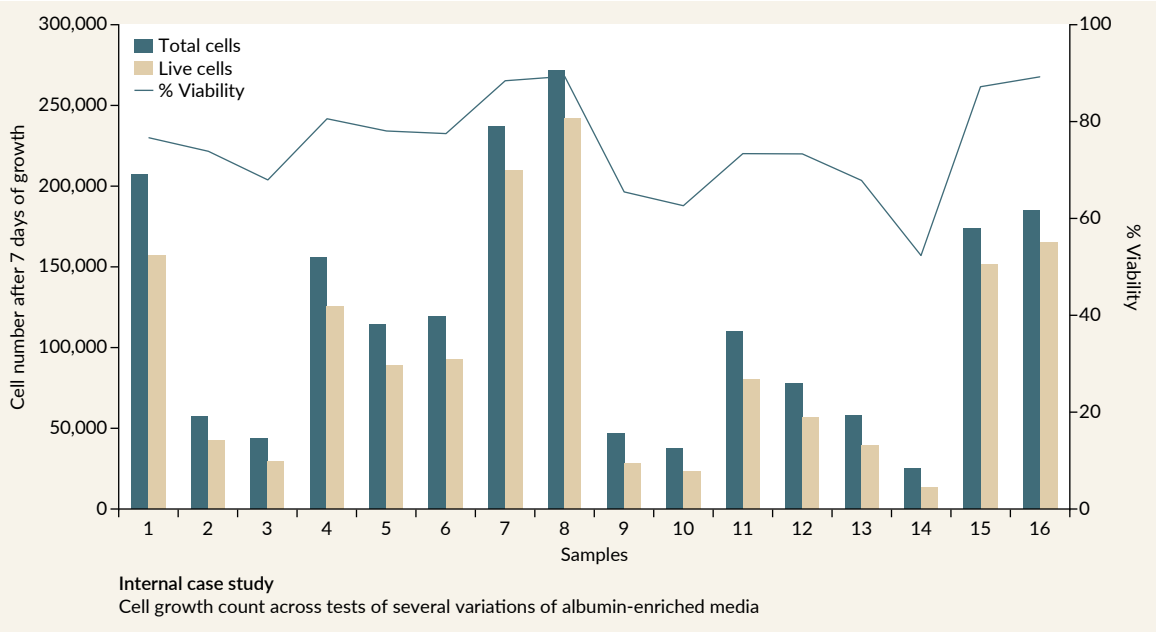
In summary, 4Cell Nutri-T Advanced medium provides superior proliferation of T cells from both healthy donors and patients, and ensures stable transduction efficiency and optimal CD4/CD8 ratios. Improved cytotoxicity and overall performance results in a more efficient process, reducing the cost per dose.

GROWTH FACTORS AND CYTOKINES TO ALLOW A SEAMLESS TRANSITION FROM R&D TO CLINICAL STAGES

Growth factors and cytokines are used throughout cell therapy manufacturing, from cell activation to expansion and differentiation. Utilizing high-quality, animal component-free cytokines is crucial for ensuring consistency, safety, and regulatory compliance in cell and gene therapy manufacturing. Sartorius produces a variety of animal component-free growth factors and cytokines available in multiple

FIGURE 3

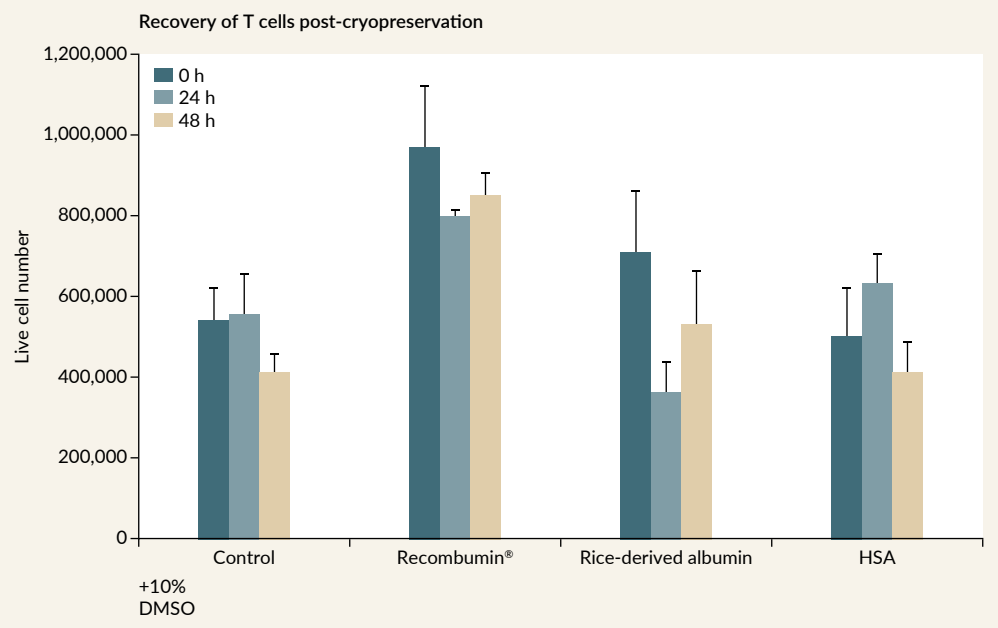
Cell growth count across tests of several variations of albumin-enriched media.



Internal case study
Cell growth count across tests of several variations of albumin-enriched media

FIGURE 4

Comparative post-thaw recovery of T cells.



grades to support a seamless transition from research to preclinical development and clinical manufacturing.

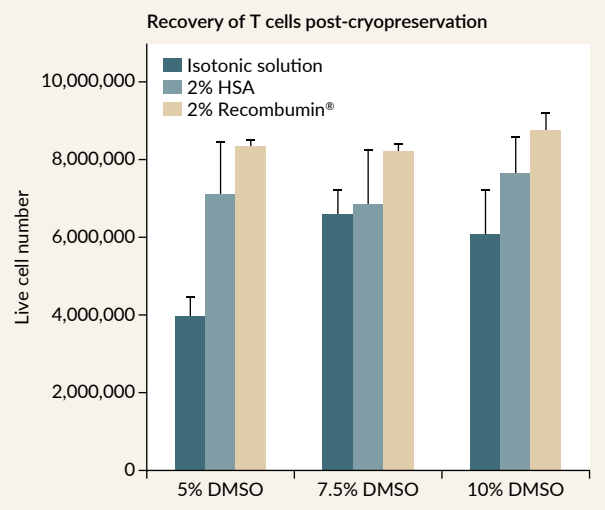
A strict animal-derived component-free (ADCF) policy is followed to ensure maximum safety of Sartorius' preclinical and GMP cytokines. As a result, no animal or human-derived components are part of any of their cytokine products. They can therefore be safely used without the need to perform time-consuming and expensive viral safety studies, thereby bringing a significant economic benefit.

Figure 11 provides a comparison of a preclinical-grade IL-2 from CellGenix® versus Proleukin®, a therapeutic IL-2, using different donors and media. The results demonstrate similar performance between the preclinical and clinical-grade IL-2, reinforcing the reliability of these products across development stages.

As these cytokines and growth factors are specifically designed with cell therapy in mind, their formulations, testing protocols, and packaging are all optimized to meet the unique requirements of this field.

FIGURE 5

Reducing dimethyl sulfoxide content for cryopreservation.



CHEMICALLY DEFINED BIOPRESERVATION SOLUTIONS

Short- and long-term storage during cell processing can pose several challenges including low post-thaw recovery.

Cryopreservation techniques are employed during cell banking, fill and finish, and long-term storage. Developers producing their own 'home-brew' cryopreservation formulations will often use human serum albumin, plasma, or serum supplemented with 5–10% dimethyl sulfoxide (DMSO). Sartorius offers chem-

FIGURE 6

Comparison of hold time and number of apoptotic cells post-thaw.

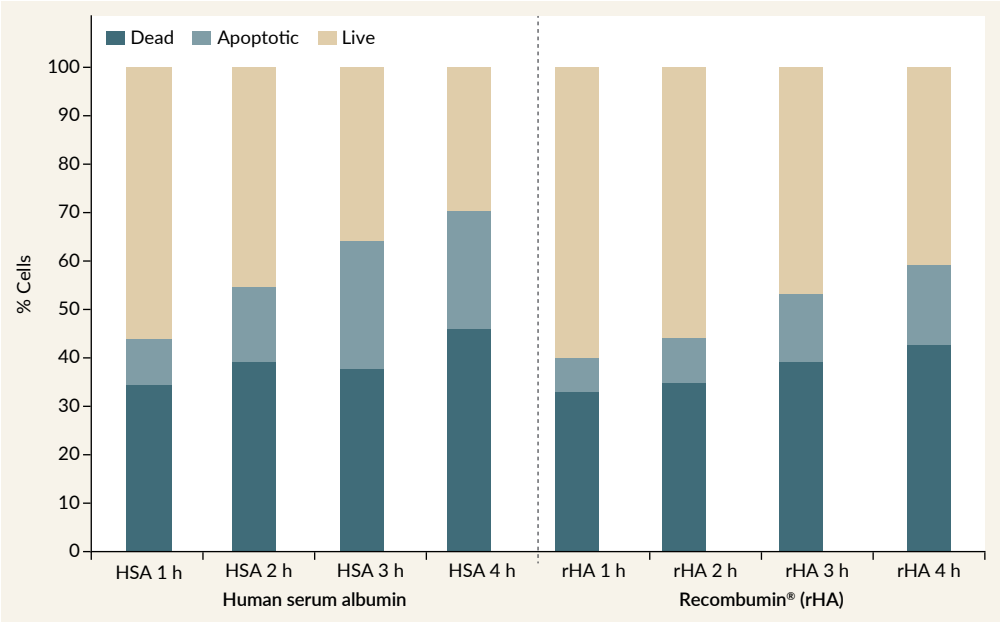
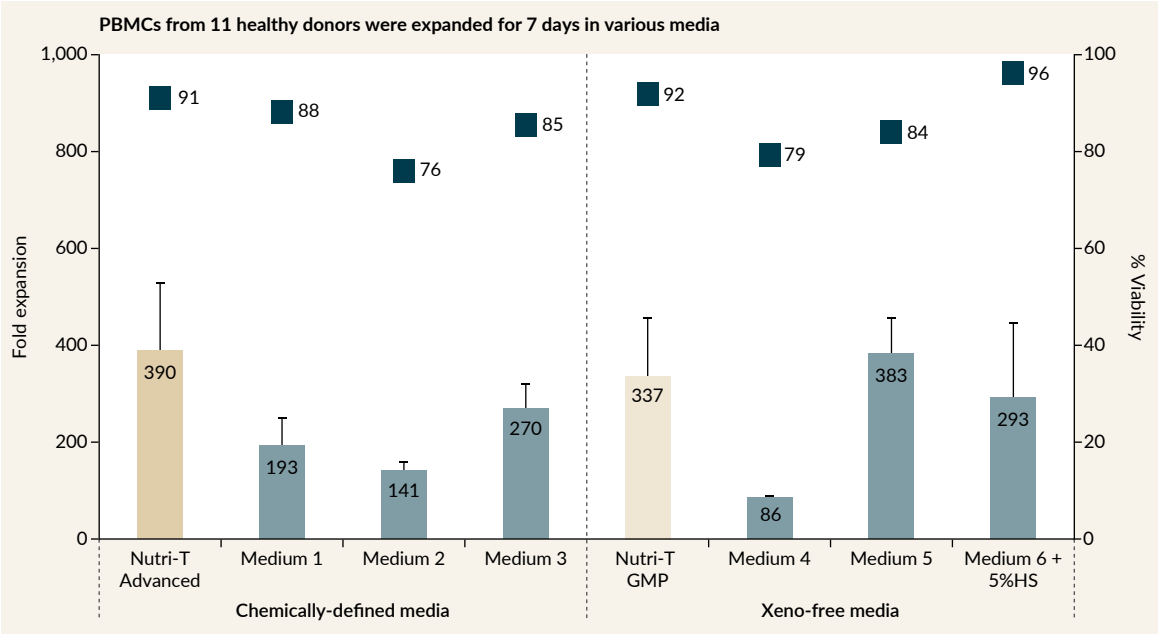


FIGURE 7

Expansion and viability with healthy donor-derived T cells.



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ically-defined, protein-free, animal component-free cryopreservation solutions NutriFreez® D5 and D10, which contain 5% and 10% DMSO, respectively. They are both manufactured in compliance with relevant cGMP guidelines and show similar results

FIGURE 8

Expansion and viability with patient-derived CAR-T cells.

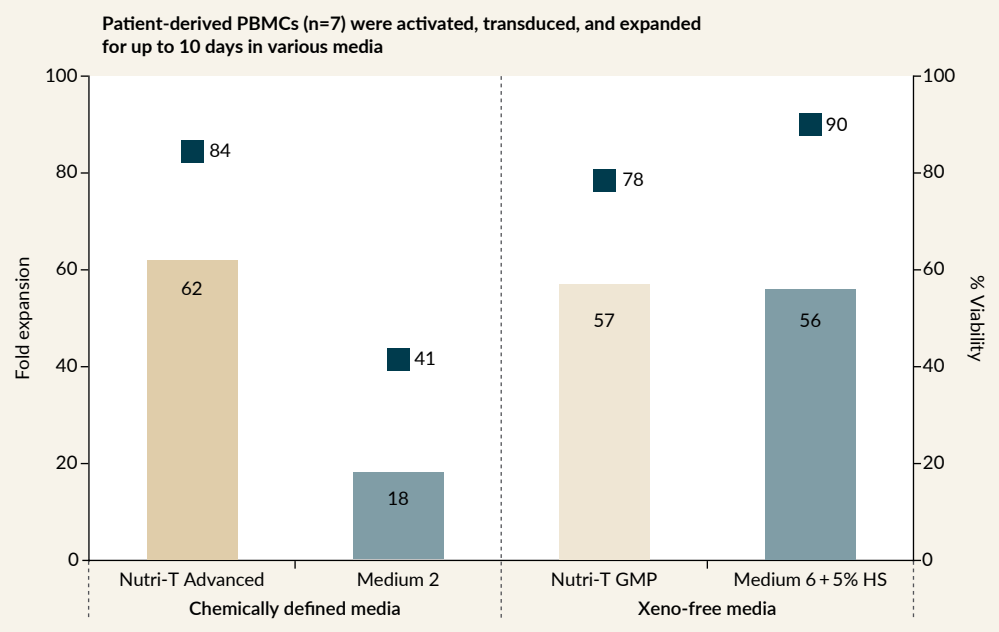

FIGURE 9

Transduction efficiency and frequency of CD8⁺ cells in patient-derived CAR-T cells cultured in G-Rex® 6M.

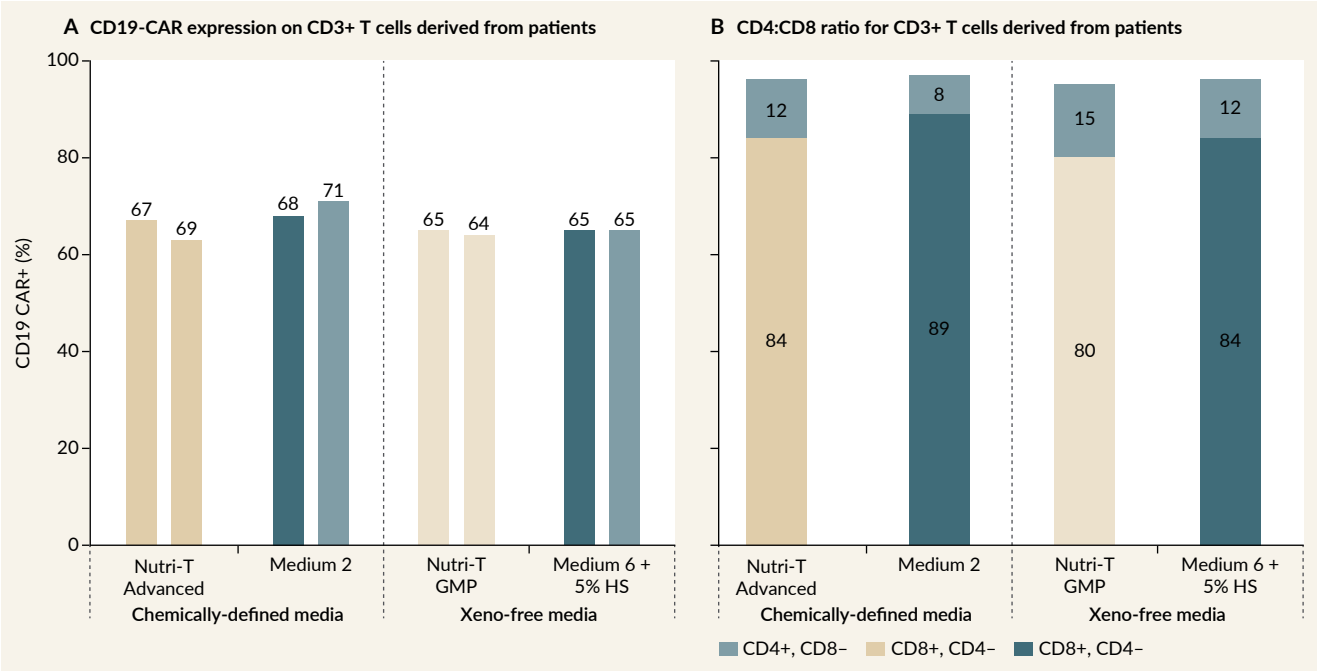

FIGURE 10

Functionality and effectiveness of CD19- CAR-T cells.

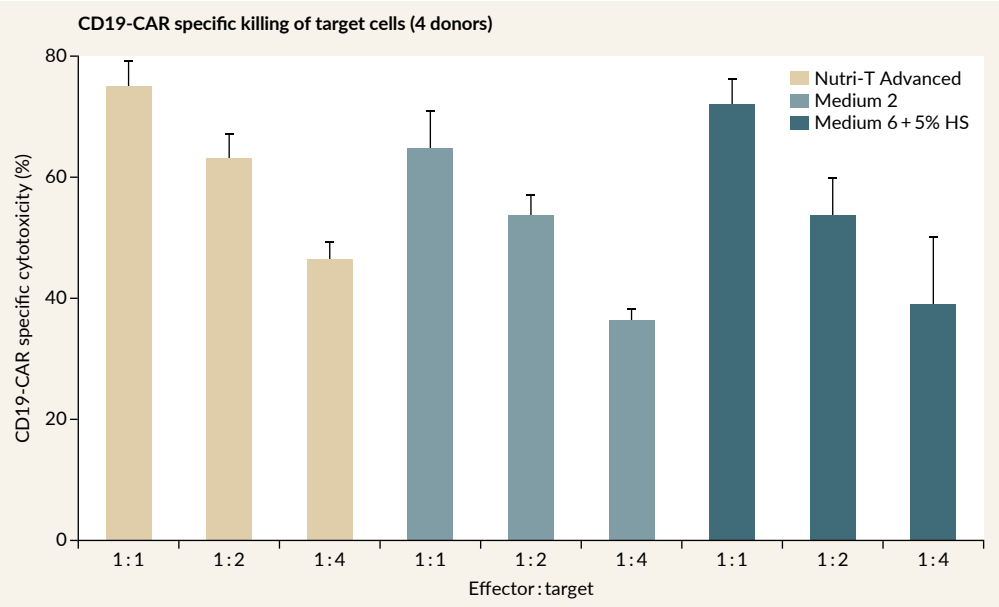

FIGURE 11

Fold expansion of peripheral blood mononuclear cells cultured using CellGenix® Preclinical IL-2 or Proleukin®.

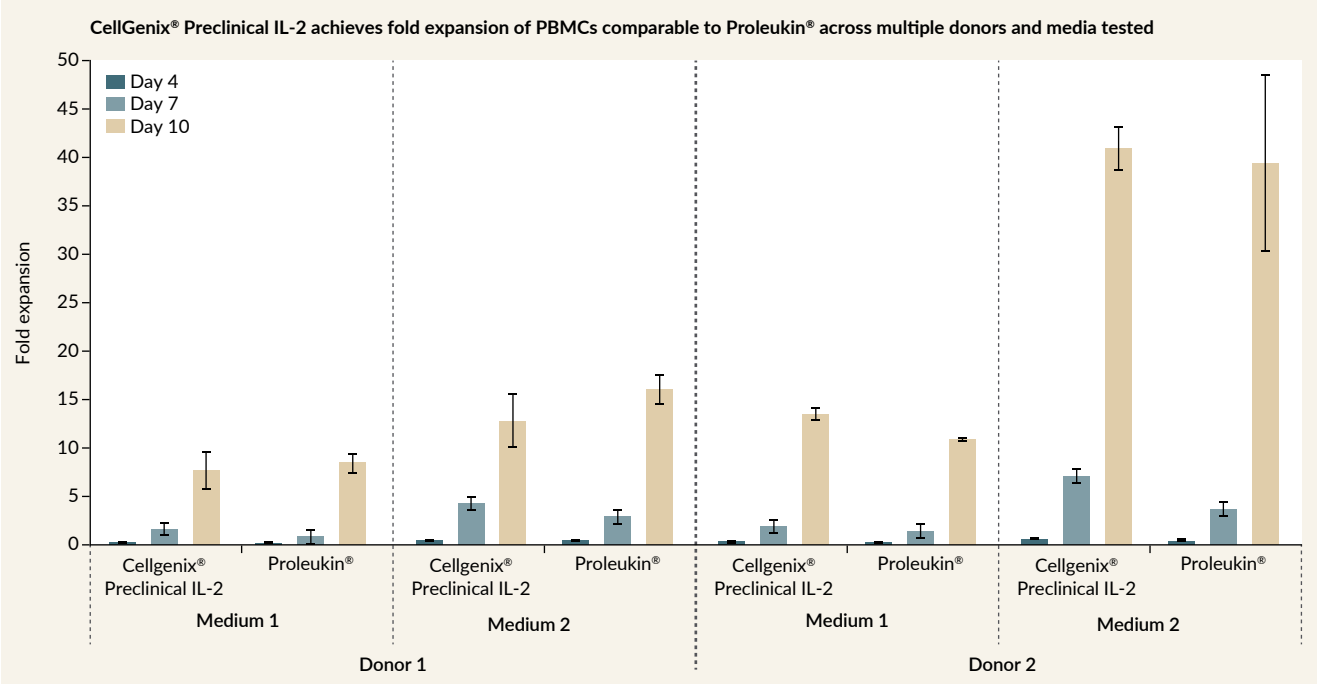

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FIGURE 12

Transduction efficiency and immunophenotype ratio in CAR-T cells 10 days post-thaw.

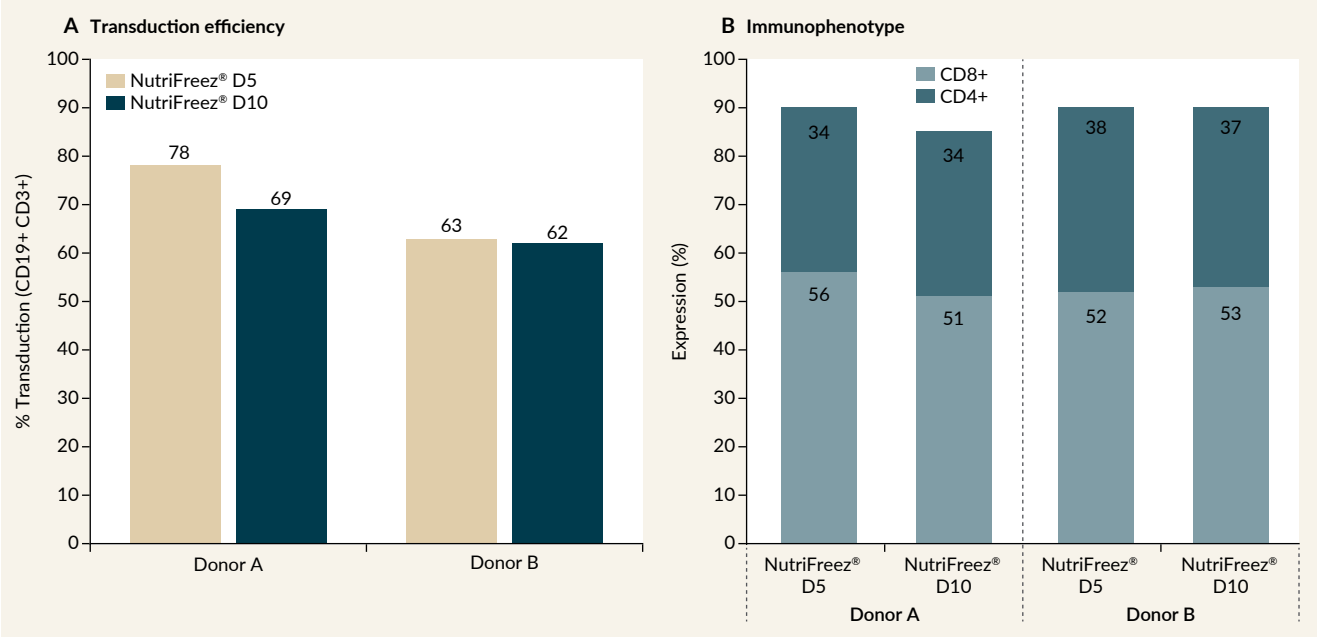


FIGURE 13

Morphology, viability, and comparative fold expansion of peripheral blood mononuclear cells with NutriStor® and competitor solutions.

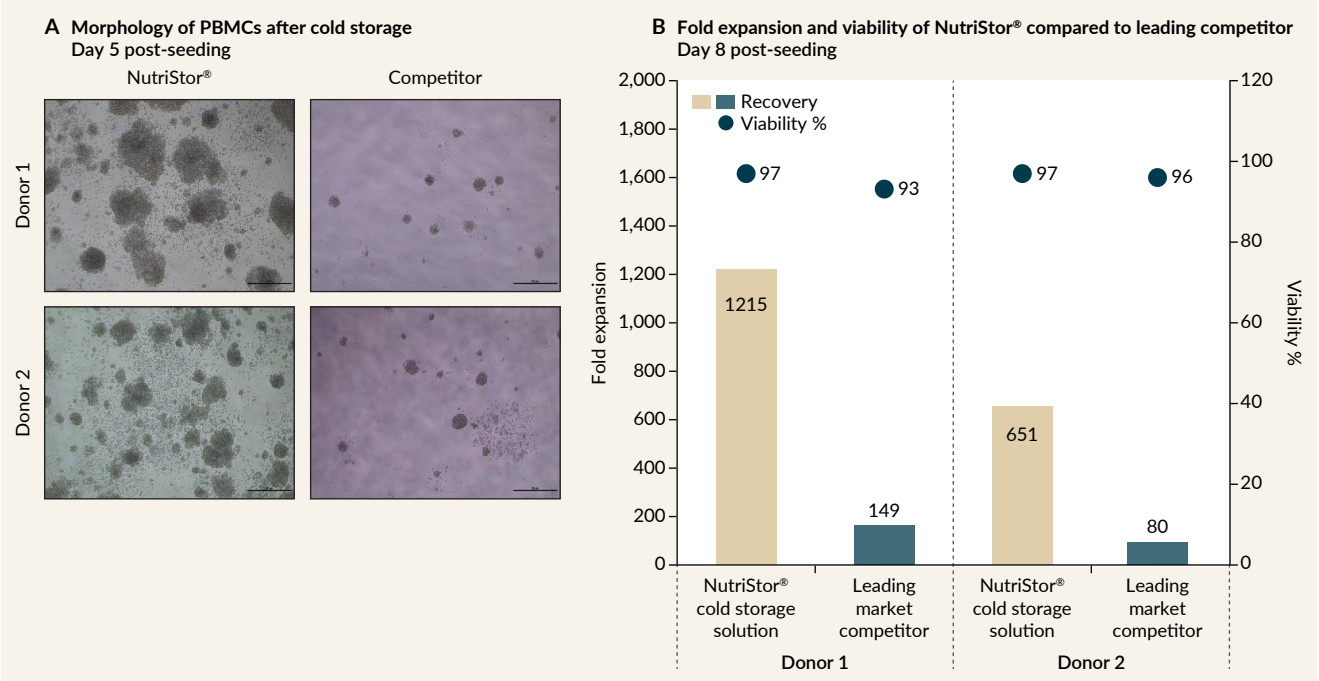
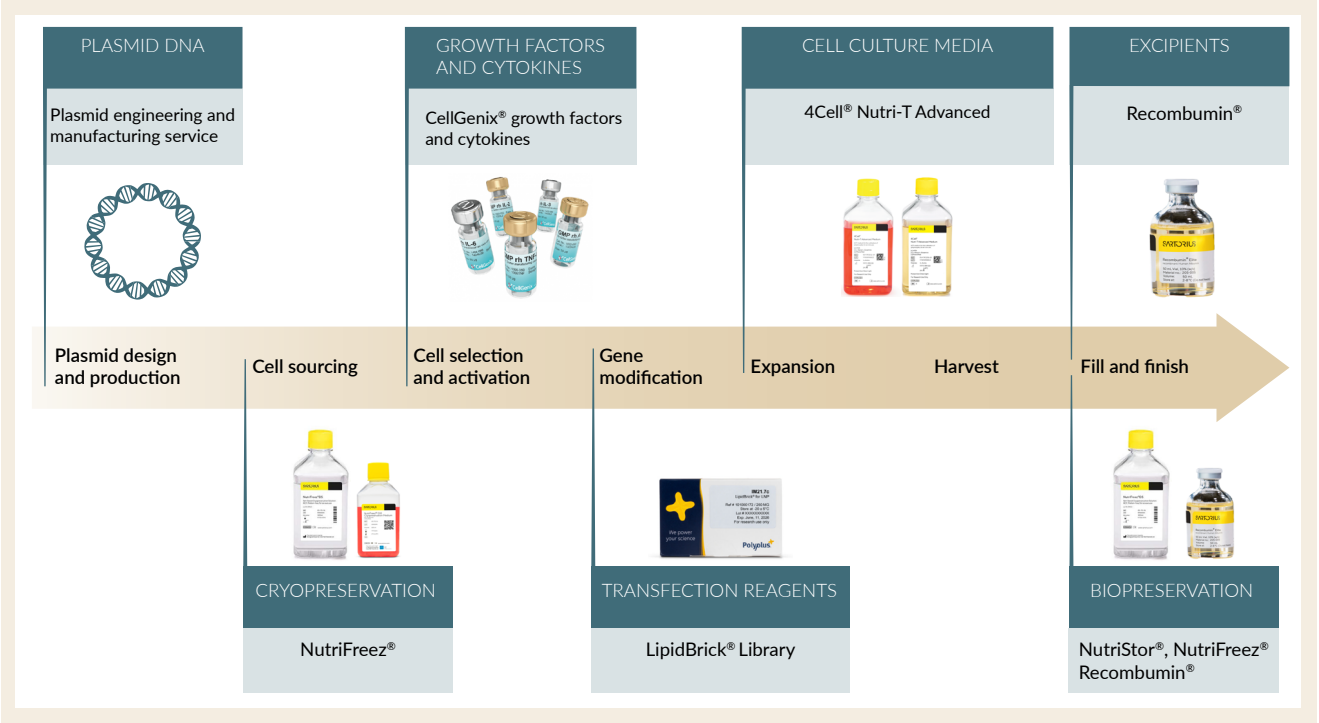


FIGURE 14

Animal-component free materials to enable safe and consistent cell therapy development and manufacturing.



in maintaining consistent transduction efficiency and immunophenotype ratio in CAR-T cells 10 days post-thaw (Figure 12). While Nutrifreez D10 is medium-based, Nutrifreez D5 is a chemically-defined, salt-based solution specially designed for freezing and thawing of cells intended for cell therapies and clinical applications.

In contrast to cryopreservation, short-term cold storage, slows down the metabolism of the cells, and therefore allows the transport of the cells without the cost-intensive and stressful effects of freeze–thaw.

NutriStor® is a chemically defined, ready-to-use cold storage solution, providing an alternative to maintain products at 2–8 °C. Used for short-term storage during expansion, differentiation, fill and finish, quality assurance, and cell transportation, this approach maintains cell viability and functionality following cold storage while mitigating freeze–thaw induced cell stress. This solution also comes with comprehensive regulatory documentation (current GMP, DMF, regulatory support file, and pre-clinical safety studies) to support patient safety.

A comparative study against a leading competitor was conducted (Figure 13) using PBMCs which were stored at 2–8 °C for 4 days and subsequently cultured. PBMCs previously stored in NutriStor demonstrated significantly better growth, higher expansion rates, and excellent viability post-storage. This highlights NutriStor's capacity to support robust cell health and functionality under non-cryopreserved conditions.

TRANSLATION INSIGHT

Transitioning to chemically defined raw materials for cell therapy manufacturing offers a number of key benefits and can offer comparable or improved results and performance compared to raw materials containing human- or animal-derived components. Working with highly defined and

consistent materials allows for optimized processes, without the variability and associated risk that comes with new batches of raw materials. Starting with the end in mind is crucial for cell therapy manufacture, as more reliable and efficient processes ultimately shorten time-to-market, help to reduce cost-per-dose, and enhance patient safety.

You can learn more about Sartorius' portfolio of animal component-free materials for cell therapy in Figure 14.

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SPOTLIGHT

Efficient non-viral engineering of immune cells for cell therapy using circular single-stranded DNA

Howard (Hao) Wu

VIEWPOINT

"The integration of precision gene editing with safe and scalable delivery systems represents a paradigm shift in how we think about and approach immunotherapy."

Cell & Gene Therapy Insights 2025; 11(1), 7–11 · DOI: 10.18609/cgti.2025.002

RESEARCH BACKGROUND

In recent years, gene-editing technologies have become central to advancements in immunotherapy, particularly in engineering immune cells for the treatment of diseases such as cancer and autoimmune disorders. However, traditional methods—especially those relying on viral vectors for gene delivery—have raised significant concerns related to safety, efficacy, scalability, and accessibility. These concerns have slowed the widespread adoption of these therapies over the globe. Recently, a Boston, MA, USA-based biotech company Full Circles Therapeutics achieved technological breakthrough to overcome many of these limitations, signaling a new era in the development of safe, scalable, efficient, and more affordable immune cell therapies.

Although CRISPR/Cas9 technology has been widely used in gene editing, enabling

efficient targeted gene knockout and single-base editing, precise and site-specific integration of large gene fragments remains a technical challenge. These current gene delivery technologies face the following issues:

- Limitations of viral vectors: including immunogenicity, which can trigger immune responses; difficulty in production, as the viral preparation process is complex and costly; payload limitations, such as the restricted gene capacity of AAV vectors; and safety concerns, as random gene integration by lentivirus and AAV can pose potential safety risks.
- Drawbacks of non-viral methods: such as low efficiency and high toxicity associated with double-stranded DNA (dsDNA), and challenges in large-scale production of linear single-stranded DNA (lssDNA).

These technological bottlenecks present significant challenges for the engineering of immune cells (such as CAR-T cells) and the clinical application of cancer immunotherapy. Therefore, there is an urgent need for a non-viral, efficient, and safe gene integration technology.

RESULTS AND CONCLUSION

The company's innovation developed a proprietary miniaturized circular single-stranded DNA (cssDNA, trademarked as C4DNA™), a non-viral technology that enables stable genomic integration without relying on viral vectors. They developed a non-viral gene writing technology (GATALYST™) platform based on the C4DNA, which has brought disruptive progress in the field of large gene fragment integration. This platform utilizes a genetically engineered phage system to produce mini circular single-stranded DNA (cssDNA) with a size of up to approximately 20 Kb. It achieves efficient and precise large gene integration through a homologous recombination mechanism.

The cssDNA non-viral vector avoids the genomic instability caused by random integration and has the potential for industrial-scale production, significantly improving manufacturing efficiency. The GATALYST™ platform can achieve efficient, precise, and safe integration of large genes in various dividing cells. It has demonstrated up to 70% knock-in efficiency in induced pluripotent stem cells (iPSCs), as well as high-efficiency site-specific integration in multiple clinically relevant primary immune cell types. The platform can edit multiple genomic loci relevant to clinical applications and is compatible with various nucleases editing systems.

Using electroporation delivery technology, cssDNA can achieve up to 50% site-specific integration of dual-targeting CAR (CD19/CD22) in CAR-T cells, exhibiting excellent anti-tumor function both *in vitro* and *in vivo*. This provides new avenues for treating relapsed or refractory multiple myeloma (RRMM).

The GATALYST™ platform, with its flexible, safe, and scalable cssDNA vector, shows great potential in the field of immune cell therapy. This platform is expected to overcome the limitations of current gene delivery methods and provide a novel non-viral solution for gene and cell therapies targeting cancer, autoimmune diseases, and genetic disorders, thus advancing the development of precision medicine.

PERSPECTIVE

The non-viral revolution: precision and safety in focus

One of the most significant challenges in the field of gene editing has been ensuring the precise and controlled integration of genetic material into the genome. Full

Circles' use of C4DNA, a miniaturized form of circular single-stranded DNA, enables targeted, accurate genomic integration. This eliminates the reliance on potentially unpredictable viral systems, providing a safer alternative for patients. The technology significantly enhances scalability, editing efficiency, and overall safety, particularly for CAR-T and CAR-NK cell therapies. These therapies, which have already demonstrated significant promise in treating cancers, could become more accessible and less expensive as the technology scales up. For autoimmune diseases where the safety bar is much higher than end stage cancers, adoption of a non-viral engineering approach is more attractive.

The company's approach directly addresses the scalability problem, which has been a major bottleneck in the widespread use of gene-edited immune cells. One of the key challenges for current therapies, such as CAR-T, is the complexity and cost involved in producing personalized therapies for each patient. Full Circles' non-viral method could lead to the development of off-the-shelf cell therapies with non-viral precise genome engineering, meaning that engineered immune cells could be produced in bulk and stored for rapid use in patients, much like other biologics, making treatments more affordable and time efficient. Full Circles is now leveraging the multiplex editing approach by C4DNA to manufacture the allogeneic CAR-NK product for autoimmune diseases.

A new path for clinical application

Full Circles' recent patent approval in Japan, a leading global market for biopharmaceuticals, is a significant milestone for the company and for the field at large. The granting of the patent underscores the technology's clinical potential, particularly in markets where gene therapies are already a focus of intense research and development. With Japan at the forefront of oncology, autoimmune disease research, and genetic metabolic disorders, the impact of this innovation could be transformative.

The granted patent, which covers the use of universal circular single-stranded DNA for targeted genomic integration, highlights the broad utility of this technology in genome editing. Importantly, this technology is designed to work in a nuclease-editor agnostic manner, a feature that improves its adaptability, programmability and target specificity.

Expanding the therapeutic landscape

The broader implications of Full Circles' breakthrough extend beyond the immediate application to CAR-T and CAR-NK therapies. The potential for using this non-viral gene-editing technology could dramatically expand the range of diseases that can be treated with engineered immune cells. By enhancing the efficacy, scalability, and cost–effectiveness of immune cell therapies, Full Circles has positioned itself to address complex and hard-to-treat conditions—such as cancers, chronic autoimmune diseases, and perhaps even genetic disorders caused by ultra-large root cause genes with scattered mutation haplotypes—that have long eluded conventional treatments.

Dr Richard Shan, the Chairman of Full Circles Therapeutics, emphasized that the company aims to make these therapies more accessible and affordable to a broader patient population. In an era where the cost of biologics and personalized medicine remains a critical barrier to widespread adoption, the scalability of Full Circles' non-viral approach offers a promising solution. By making cell therapies off-the-shelf, the company could facilitate faster and more efficient deployment of treatments, ultimately accelerating the clinical translation of these breakthrough technologies.

Looking ahead: the path to widespread adoption

The recent recognition from Novo Nordisk Bio innovation Hub, which awarded Full Circles the 'Pathbreaker' award, underscores the company's commitment to collaborating with industry partners and accelerating the accessibility of breakthrough therapies. The award is a testament to the importance of forging strategic partnerships to drive the next generation of therapeutic innovation. By focusing on patent licensing and collaborative development, Full Circles aims to expand the reach of its technology and increase its impact in the global healthcare landscape.

Ultimately, Full Circles' non-viral immune cell engineering technology could become a cornerstone in the future of cell and gene therapy, enabling a broader range of patients to benefit from cutting-edge treatments. The integration of precision gene editing with safe and scalable delivery systems represents a paradigm shift in how we think about and approach immunotherapy. With further advancements and clinical validation in the near future, we are confident that this technology could play a key role in shaping the next generation medical treatment for a wide array of diseases.

This VIEWPOINT is based on the following original publication: Xie K, Starzyk J, Majumdar I, *et al.* Efficient non-viral immune cell engineering using circular single-stranded DNA-mediated genomic integration. *Nat. Biotechnol.* 2024; published online Dec 11. https://doi.org/10.1038/s41587-024-02504-9.

BIOGRAPHY

Howard (Hao) Wu has almost 20 years of experience in gene editing technology and new drug discovery. He is specialized in overseeing R&D programs, new lab and research team set up in the biotech start-ups. Dr Wu is the co-founder and CSO of Full Circles Therapeutics, Cambridge, MA, USA where he is dedicated to developing curative gene editing based gene/cell therapy. He is responsible for generating revenue through collaboration with MNC and biotech partners. Before founding Full Circles Therapeutics, Dr Wu was leading multiple discovery biology programs and disease prioritization in the genetic disease space at Fulcrum Therapeutics Inc. (NASDAQ:FULC), a small molecule drug discovery biotech company. He had been with the company through the full development phases starting from the start-up, expansion, until post-IPO development, during which he led a cross-functional team for portfolio disease selection and prioritization of multiple disease programs including neuromuscular disease, cardiac disease, hematological and metabolic diseases. Before joining Fulcrum, Dr Wu was a Senior Research Fellow at Whitehead institute, MIT. His research focused on neurological disorders utilizing a combination of CRISPR/Cas9 mediated genomic and epigenomic editing technology and stem cell technology. He did his PhD in Biochemistry and Structural Biology at Hongkong University of Science and Technology (HKUST), Hong Kong and Bachelor's degree in Chemistry from Fudan University, Shanghai, China before he did his postdoctoral research at Johns Hopkins University School of Medicine, Baltimore, MD, USA and Howard Hughes Medical Institute, Chevy Chase, MD, USA. Dr Wu has more than 30 journal publications, patents, and research and industry grants. For his work, he has received a fellowship award from the Human Frontier Science Program (HSFP) and the NARSAD Young Investigator Award. He was also awarded the Alfred Blalock Young Investigator Award from JHMI and President's award from Whitehead Institute, MIT.

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EXPANDING CELLULAR IMMUNOTHERAPY

SPOTLIGHT

Solid tumor treatment: how can we achieve therapy efficacy and persistence in the next frontier of cell therapies?

INTERVIEW

"We aim to overcome the immune suppression that cancer can cause, so our therapeutic cells can effectively target the cancer cells."

Though there have been significant advancements in adaptive cellular therapies, researchers continue to face challenges in enhancing efficacy and targeting solid tumors. Abi Pinchbeck, Editor, BioInsights, speaks with **Sebastian Kobold**, Professor of Experimental Immuno-Oncology, LMU University Hospital, about the potential of emerging cellular immunotherapy strategies and approaches to overcoming the barriers in current treatment paradigms.

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Q What are you working on right now?

SK The primary interest of my lab is to discover and develop novel strategies to overcome limitations in adoptive cellular therapies. Historically, most of our work has focused on T cells, but we have expanded to explore other cell types.

In essence, my lab's efforts revolve around three main pillars. First, we are exploring how to target cancer cells more efficiently and selectively. We are working on both methods and receptor types that allow us to target cancer cells in a highly selective way, ideally sparing healthy cells and enabling us to reach a wide range of cancer cells.

The second area we focus on is guiding T cells to areas of interest. In our case, this is primarily the cancer or its metastases. We are developing strategies to selectively direct T cells toward these sites, with one prominent example being our work on chemokines and chemokine receptors, which is a major area of interest for my team.

Lastly, we are addressing the issue of dysfunction or immune suppression at the cancer site. We aim to overcome the immune suppression that cancer can cause, so our therapeutic cells can effectively target the cancer cells. To achieve this, we use engineering to design new receptors and modify cellular components to enhance desired functions or prevent suppressive effects at the cancer site.

Q What are the current key challenges preventing the success of CAR-T cell therapies in solid tumor indications?

SK There are, in my view, three major scientific or biological challenges to face when designing or developing a therapy for solid cancers.

The first, which is a more global drug development concept, is how to deliver the drug to the target site. Since we are dealing with therapeutic cells, which are living drugs, how can we bring these cells to the cancer site, metastases, or any critical area for treatment? This requires a selective, specific approach. If the cell therapy does not reach the necessary site, there is virtually no chance it will be effective. This concept applies to any drug: if it does not reach its action site, it will not effectively treat the disease.

The second biological challenge is enabling T cells or immune cells to recognize cancer cells properly. If these cells cannot identify their target, they cannot be effective. This issue is especially challenging in cellular therapies for solid tumors due to disease heterogeneity. Solid tumors evolve over long periods, allowing for multiple mutations and evolutionary changes before treatment is possible. We still lack a robust solution to address this heterogeneity while targeting most or all cancer cells and sparing healthy cells.

The last challenge is overcoming immune suppression at the cancer site. This is, perhaps, the most difficult challenge. Suppression can happen through direct physical interactions—physical, soluble, or otherwise—or indirectly, as immune cells may lose function due to biological limits. These cells were not necessarily designed to perform the sustained functions we expect of them in therapy. This suppression is often driven by cancer but can also result from the natural life cycle of an immune cell. We need to address both aspects when designing therapies; otherwise, even the most specific and selective therapy will not perform well if it loses functionality at the target site.

Ultimately, these biological challenges are crucial to solving the problem of cell therapies in solid tumors. But we also face technical, logistical, and financial challenges in making these therapies viable in such indication. While I am confident that solving the biological issues will eventually lead to solutions for these other hurdles, it is clear that we do not yet have all the answers.

Currently, cell therapies are costly, labor-intensive, and time-consuming to produce. Manufacturing these products requires complex logistics to transport cells to and from

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patients, high-quality reagents (which are often in short supply), and adequate production facilities. These factors can create access barriers for patients, especially in cases where the disease progresses too quickly to allow for cell collection or expansion.

This complexity results in a high price tag, with cell therapies estimated to cost $300,000–450,000 per treatment, excluding hospitalization and other care expenses. If we hope to use cellular therapies for solid tumors, which account for 90% of cancers worldwide and affect millions each year, we need a different logistical and financial model. At the current cost and with existing logistical constraints, widespread application is not feasible. Therefore, we need solutions that make cell therapy production, availability, and process development more efficient and affordable for health systems globally.

Q How can we improve efficacy and persistence in solid tumors for CAR-T cell therapies and other cellular immunotherapies?

SK The potential of cellular therapies lies in overcoming several key challenges to achieve their promise—providing a one-time treatment that delivers lasting therapeutic effects. As I mentioned, current issues include technical, logistical, and financial challenges as well as biological ones. Re-dosing or giving additional doses of the same therapy is not typically effective if the initial treatment fails, as this likely indicates an inherent biological mismatch. However, in some specific cases, repeated dosing has shown individual success, though it is not broadly applicable.

An alternative approach may involve targeting earlier stages of cancer when the immune system is less compromised and more responsive. Evidence from immunotherapy trials, particularly with checkpoint inhibitors in diseases like melanoma, suggests that treating patients at earlier stages can enhance response rates. In hematologic cancers, moving CAR-T therapies to earlier treatment lines has shown promise in improving outcomes. Finally, a more experimental approach that many researchers are exploring is enhancing cellular efficacy and longevity through genetic and cellular engineering, potentially enabling therapies to overcome immunosuppressive barriers and extend the cells' therapeutic lifespan.

Q Can you go into more detail surrounding the add-on strategies for cellular therapies in solid tumors? What approaches are the Kobold Lab investigating, and where is promise being seen?

SK In the field, we are seeing clinical evidence that cellular therapeutics may be effective for solid tumors. For a long time, clinical successes in the

"One of the most exciting developments is that we now have two approved products for solid tumors in the USA, which, though not yet approved in Europe, indicate clear evidence of efficacy."

solid tumor space when treating patients with cells have been elusive. However, this has changed significantly over the last 2–3 years, after nearly two decades of limited breakthroughs.

One of the most exciting developments is that we now have two approved products for solid tumors in the USA, which, though not yet approved in Europe, indicate clear evidence of efficacy. One of these is the use of tumor-infiltrating lymphocytes (TILs) for melanoma patients who are refractory to immune checkpoint inhibitors. Two products have been successful here: the first is an academically developed TIL therapy from the Netherlands Cancer Institute (NKI) and National Center for Cancer Immune Therapy (CCIT) in Copenhagen. Their Phase 3 randomized trial met its primary endpoint of progression-free survival, with indications that overall survival may also be significantly improved. The second product is a commercial TIL therapy approved in the USA, which has shown similar results and underscores that cell-based immunotherapies can benefit even those patients who have failed checkpoint inhibition.

Another promising development is the approval of a T cell receptor-engineered cellular product targeting MAGE-A4 in refractory synovial sarcoma. This product, approved in the USA in the summer of 2024, is based on positive results from a Phase 2 clinical trial, representing a breakthrough for patients who previously had few or no effective options.

There are two other notable clinical achievements, though not yet approved, that are worth mentioning. One is a GD2-specific armored CAR, with added cytokine support and a safety switch, which was developed within the pediatric oncology field for neuroblastoma. This CAR-T therapy has delivered impressive response rates, including complete remissions in young patients who had no remaining treatment options. The other exciting advance is a CAR therapy combined with an mRNA vaccine, targeting claudin, which has shown promise in refractory testicular cancers. This combination suggests that cellular therapeutics could have a broad impact across different solid tumor types.

In our lab, we are working on three key areas. First, we are investigating chemokine receptors to improve T cell recruitment to tumors by restoring a match between tumor-expressed chemokines and the receptors on T cells. We have demonstrated that this approach can enhance T cell recruitment to tumor tissues and support therapeutic effects across a wide range of cancers, and we are now moving toward clinical development. Second, we are using computational techniques on large datasets to identify cancer-selective antigens, which led us to discover new targets in acute myeloid leukemia. We have already designed CARs targeting these antigens and confirmed their potential in a range of models, including patient-derived ones, and we are eager to take this into clinical trials. Finally, we are addressing immune suppression by engineering fusion receptors to convert suppressive signals into stimulatory ones. One prominent example is our PD-1/CD28 fusion receptor, which transforms PD-1's inhibitory signal into a CD28 costimulatory signal. This strategy, patented with our US partner, has already advanced to a Phase 1/2 clinical trial, showing the potential of modifying immune suppression pathways to enhance the efficacy of cellular therapeutics.

Q What technologies are used to modulate the tumor microenvironment and how can we overcome the associated physical, chemical, and biological challenges?

SK To begin, it is helpful to differentiate between technologies that can be applied to the therapeutic cells themselves and those applied directly to the tumor or patient environment. Focusing on cell engineering, especially for therapeutic applications, we have seen a remarkable expansion in techniques over recent years. This includes methods for receptor engineering—encompassing protein biochemistry, receptor design, and targeting strategies—to direct therapies to specific cell types and regions within the genome. Additionally, different delivery methods, from viral to non-viral approaches, use DNA, RNA, or nucleases to edit target cells precisely.

Beyond cell targeting, we are also exploring technologies that allow selective control over cells, such as on-off switches or adaptable modules that can manage or even remove cells if needed. This list is far from exhaustive, but it highlights some of the diverse techniques currently in use or under development for cell modification.

In contrast, the technologies available to directly modulate the patient's environment or tumor are in a much earlier stage. Traditionally, approaches like chemotherapy or radiation—applied either locally or systemically—are more generalized, though still among the best options for preparing a patient's environment to support subsequent therapeutic cell application. However, newer advancements are emerging, such as different forms and delivery depths of radiation, as well as small molecules or agents that target specific pathways locally or systemically.

It is essential to explore these options further in the context of cellular therapies, as traditional drugs (like tyrosine kinase inhibitors developed for oncogenic signaling) do not necessarily translate seamlessly to cellular therapeutic settings. While there is a promising range of strategies in development, much remains to be learned and innovated to refine these approaches effectively.

Q CAR-T cell therapies have seen much more success in liquid cancers including leukemias. Can you assess the state of affairs here?

SK In the field of hematology, CAR-T therapies have firmly established their place, with seven approved products currently available. Additionally, there are numerous other products in advanced stages of regulatory development, depending on the region of the globe. Many of these upcoming therapies target similar indications, such as B-cell malignancies, plasma cell disorders, or myeloma, and are nearing approval.

However, I am uncertain about the true innovation offered by some of these new therapies, as many still target the same antigens—namely CD19 and B-cell maturation antigen (BCMA)—that existing products already address. One significant trend we have observed is the push to use CAR-T therapies in earlier lines of treatment. Although not a large number of trials have explored this shift, those that have are showing promising results. These trials suggest that CAR-T therapies can maintain their strong efficacy even when used earlier in the treatment pathway. In some cases, these early-line CAR-T treatments may

even outperform conventional therapies, potentially reducing the need for patients to undergo the more burdensome traditional treatments before becoming eligible for CAR-T.

That said, outside the current success seen in B-cell malignancies and myeloma, the effectiveness of CAR-T therapies in other hematologic conditions—like acute myeloid leukemia—has been limited. This highlights a significant opportunity for improvement and innovation in hematology, as the success seen in B-cell-related cancers has not yet been replicated across other indications.

Q What are your key goals and priorities for the next 12–24 months?

SK One of our current initiatives is a project in Germany called the BAYCELLator, the Bavarian Cell Therapy Catalyst. This collaborative effort aims to accelerate the transition from exploratory research to clinical development by creating a plug-and-play system. The goal is to leverage established production tools and protocols, adapting them to specific research concepts. While this approach still demands significant work and funding, we believe it has the potential to speed up development timelines here in Germany.

In addition to this, a major objective is to broaden our focus to include more diseases and strategies on a larger scale. By doing so, we hope to address some of the limitations we previously identified and push the boundaries of what is possible in cell therapy.

BIOGRAPHY

Sebastian Kobold studied medicine in Homburg, Zurich and Bordeaux. He completed his doctoral training in tumor immunology with C Renner in Zurich and Homburg. Dr Kobold did his residency training in hematology, oncology and clinical pharmacology in Hamburg and Munich. Since 2014, he is a board-certified clinical pharmacologist and immunologist and serves as attending physician at the university hospital of the LMU, Munich, Germany. Dr Kobold trained as a post-doctoral fellow in Hamburg (D Atanackovic), Munich (S Endres) and Boston (K Wucherpfennig). In 2019, he was appointed Professor of Medicine and Experimental Immuno-oncology at the LMU Munich and as Deputy Director of the Division of Clinical Pharmacology at the University Hospital of the LMU. The research of Professor Kobold is focused on developing innovative immunotherapeutic strategies utilizing T cells for cancer treatment. His research has been internationally recognized with among others the Ernst-Jung Career Advancement Award, the Vincenz Czerny Award for cancer research, the Johann-Georg-Zimmermann Award for cancer research and more recently the Wilhelm Vaillant Award for Medical Research. Dr Kobold also serves as academic editor to the *Journal for the Immunotherapy of Cancer*, *ESMO IOTECH*, *Frontiers in Immunology*, and *Frontiers in Oncology* and reviews for international leading journals and grant funders including the *Nature* and *Science* publishing groups.

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