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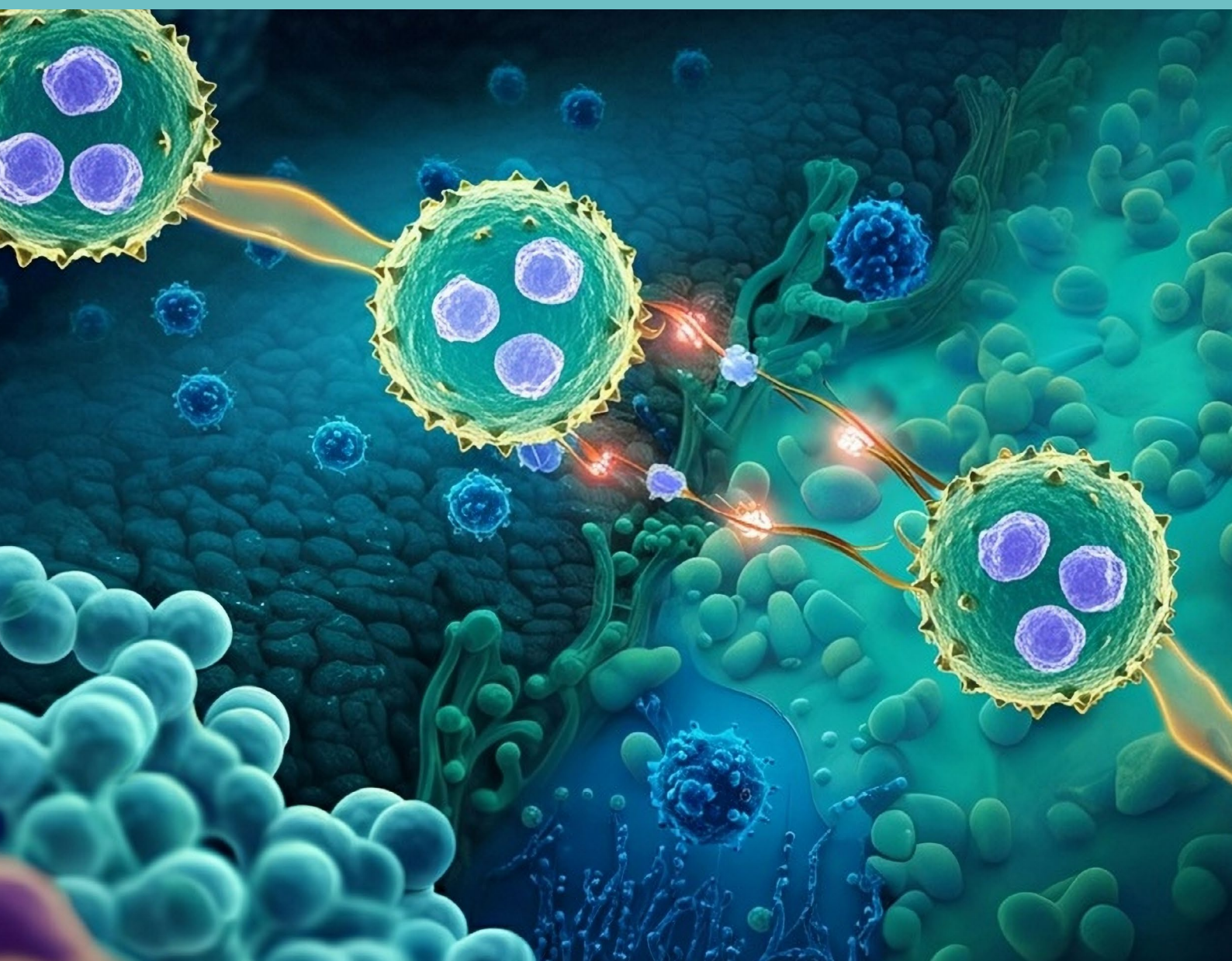
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E L E G E N

Cell therapy (beyond CAR-T) and opportunities for new medicines





Diana Spencer, Senior Digital Content Editor, DDW

Cell therapy is an area of significant financial growth. Precedence Research places the market size at \$7.43 billion in 2025 and forecasts that it will reach around \$47.72 billion by 2034, accelerating at a CAGR of 22.96%.

The market has so far been led by chimeric antigen receptor T cell (CAR-T) therapies, which have been demonstrated to enable complete disease remission, omitting the need for additional treatment. However, practical limitations and safety concerns have so far restricted the potential of these breakthrough treatments, and many companies are now investigating other types of adoptive cell therapy. These include tumour-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, regulatory T cell (Tregs) therapy, and natural killer (NK) cell therapy.

To date, cell therapy drug discovery has been mostly

focused on oncology, particularly haematological malignancies, but there are now cell therapies in development in other disease areas, including autoimmune and neurodegeneration. Unfortunately, pharma investment has not necessarily translated into regulatory success. While there are a large number of ongoing clinical trials, there are currently just 14 cell therapy products approved by the US Food & Drug Administration (FDA). There is steady progress, however, and 2024 saw the first FDA approvals for cell therapies to treat solid tumours.

As well as safety concerns, the slow nature of the process and a simple lack of manufacturing capacity are other limiting factors that are currently preventing patient access to cell therapies. However, many initiatives and strategies are currently in place to tackle these, including enhancing manufacturing capacity, reprogramming cells *in vivo* to simplify the manufacturing process, or using off-the-shelf allogeneic therapies that increase production scalability, so it is hoped that the promise of cell therapies could soon be realised on a wider scale.

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Cell therapy: A global market overview

With significant investment in development and manufacturing, the cell therapy market is expected to boom in the next five years.

The cell therapy market has so far been led by chimeric antigen receptor T cell (CAR-T) therapies, which leverage the body's own immune system to target and eliminate cancer cells. CAR-T therapies have been demonstrated to enable complete disease remission, omitting the need for additional treatment. Yet, despite investment and enthusiasm from the industry, practical limitations have so far restricted the potential of these breakthrough treatments.

Strong growth driven by demand for personalised medicine

Cell therapy is an area of significant financial growth. Precedence Research places the market size at \$7.43 billion in 2025 and forecasts that it will reach around \$47.72 billion by 2034, accelerating at a CAGR of 22.96%¹. This will likely be driven by the growth in cancer rates and the demand for more effective and more personalised cancer treatments.

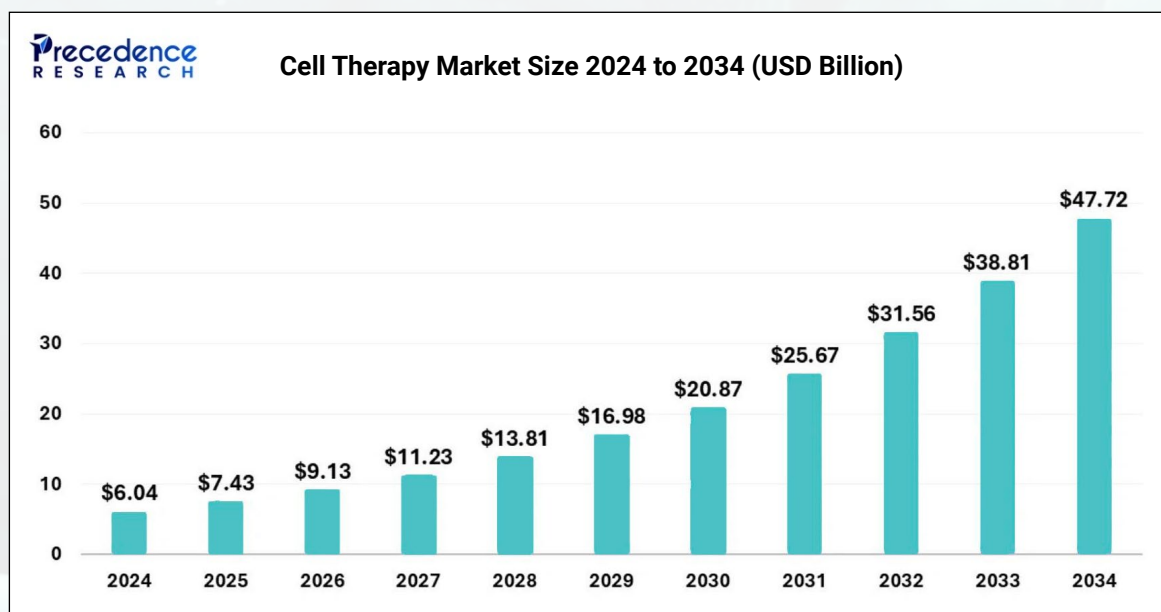
The market has also been boosted by the development of cell banking facilities and the ensuing growth in cell manufacturing, storage, and characterisation which have enhanced the industry's ability to handle large volumes on a global scale².

Furthermore, more companies are now offering cell therapy characterisation research and analysis services which is advancing cell-based therapeutics manufacturing.

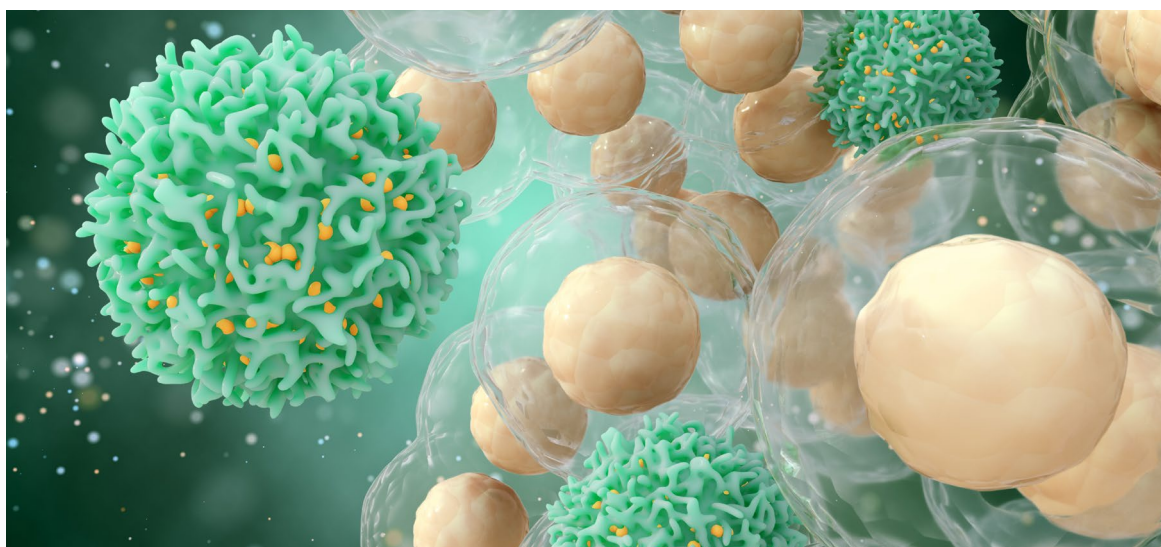
Although the market is currently dominated by oncology, Grand View Research predicts that the musculoskeletal disorders segment will witness significant market expansion in the forthcoming years². In 2024, 58% of cell therapy clinical trials were focused on non-oncology applications³.

According to analysts ResearchAndMarkets, in isolation, the global CAR-T therapy market is estimated to grow from \$4.6 billion in 2025 to \$15.2 billion by 2035, at a CAGR of 11.4% during the forecast period⁴. Close to 1,000 clinical trials related to CAR-T cell therapies have been registered in the past eight years, while over 230 companies are currently engaged in their development for the treatment of different oncological and non-oncological disorders.

The report also reveals that CAR-T cell therapies, with over 1240 preclinical / clinical product candidates, represent one of the most active segments in



Source: <https://www.precedenceresearch.com/cell-therapy-market>



pharmaceuticals. Close to 75% of these candidates are autologous in nature, and CD19 and BCMA have emerged as the most popular target antigens⁴. Grand View Research expects that the allogeneic cell therapy segment will experience substantial growth in the market from 2024 to 2030, with 542 active allogeneic CAR-T agents in the global pipeline².

In terms of disease segment, the current market for CAR-T therapies is segmented into acute lymphoblastic leukaemia, diffuse large B-cell lymphoma, follicular lymphoma, large B-cell lymphoma, mantle cell lymphoma, multiple myeloma and other haematological malignancies⁴. ResearchandMarkets predicts that the market for therapies designed to treat large B-cell lymphoma is projected to grow at an annual growth rate of 14% up to 2035.

Based on target antigen, the CAR-T market is segmented into CD19, BCMA, CD20, CD19 / CD22 and others. The report estimates that the segment for CD19 is likely to capture more than 65% of the overall market in the coming years, which is attributed to the success of CD19 targeting therapies, such as Kymriah (tisagenlecleucel) and Yescarta (axicabtagene ciloleucel)⁴.

Big pharma competes to lead the market

Analysts expect continuous financial support from investors to propel the steady growth of cell therapy in the mid to long-term, a prediction which is supported by the large number of recent industry partnerships and acquisitions in this sector. The race to be the leader in cell therapies has led many of the big pharma companies to pursue acquisitions of cell therapies and cell therapy developers.

In one of the biggest deals of 2024, Roche bought allogeneic CAR-T specialist Poseida Therapeutics

for \$1.5 billion. The move built on the existing partnership between the two companies which has been in place since 2022. The target of the acquisition was lead programme, P-BCMA-ALLO1, an allogeneic CAR-T therapy targeting B-cell maturation antigen (BCMA), which has received Orphan Drug Designation and Regenerative Medicine Advanced Therapy designation for relapsed/refractory multiple myeloma (MM). Poseida's second clinical programme in Phase I is P-CD19CD20-ALLO1, an allogeneic dual CAR-T in B-cell malignancies and potentially multiple sclerosis and systemic lupus erythematosus. Levi Garraway, Head of Product Development and Chief Medical Officer at Roche, comments: "This exciting acquisition will allow us to drive further progress in allogeneic cell therapy while leveraging the successful existing partnership with Poseida. This acquisition builds on our joint progress to catalyse the development of potentially first and best-in-class cell therapies in oncology, immunology and neurology."

The trend has continued into 2025. March saw Bristol Myers Squibb (BMS) acquire cell therapy developer 2seventy bio, manufacturers of Abecma, also for MM, for approximately \$286 million, and AstraZeneca's purchase of *in vivo* cell therapy company EsoBiotec. Susan Galbraith, Executive Vice President, Oncology Haematology R&D, AstraZeneca, explains the rationale for the acquisition: "We are excited about the acquisition of EsoBiotec and the opportunity to rapidly advance their promising *in vivo* platform. We believe it has the potential to transform cell therapy and will enable us to scale these innovative treatments so that many more patients around the world can access them. EsoBiotec will accelerate and expand the impact of our recent investments and marks a major step forward in realising our ambition to harness the full potential of cell therapy."

The buoyancy of the cell therapy market is also reflected in the large-scale financings of the last few years, most recently Arsenal Biosciences, which closed an oversubscribed \$325 million Series C financing round towards the end of 2024 to support its development of CAR-T therapies for solid tumours. The company's pipeline is based on its proprietary T cell engineering technology, including logic gating, and includes potential therapies for ovarian, kidney, and prostate cancers, as well as other solid tumours being co-developed through a collaboration with Bristol-Myers Squibb. Its second T cell product candidate, AB-2100, recently entered the clinic in a Phase I/II clinical trial for clear-cell renal cell carcinoma (ccRCC) and has been granted Fast Track designation by the US Food and Drug Administration (FDA).

Regulatory success for cell therapies

Despite the large number of ongoing clinical trials, there are currently just 14 cell therapy products approved by the US Food & Drug Administration⁵. There is steady progress, however, and 2024 saw the first FDA approvals for cell therapies to treat solid tumours. The most recent CAR-T therapy to receive approval was Autolus' obecabtagene autoleucl (Aucatzyl), a CD19-directed genetically modified autologous T cell immunotherapy, for adults with relapsed or refractory B-cell precursor acute lymphoblastic leukaemia (ALL). It was the first CAR-T therapy approved by the FDA with no requirement for a Risk Evaluation Mitigation Strategy (REMS) programme, however, the FDA has added a boxed warning for cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and T cell malignancies to the prescribing information.

There has been more regulatory activity in the last 12 months in other types of cell therapy. In August 2024, Adaptimmune Therapeutics' Tecelra (afamitresgene autoleucl) became the first engineered cell therapy for a solid tumour cancer approved in the US. It was also the first new therapy option in more than a decade for synovial sarcoma, which is a rare, soft tissue cancer that most commonly impacts young adults. In the same month, the FDA approved Lymphir (denileukin diftitox-cxdI) for the second-line treatment of r/r cutaneous T-cell lymphoma (CTCL), a novel immunotherapy and the only CTCL therapy that targets the interleukin-2 (IL-2) receptor found on malignant T-cells and Tregs.

However, even once these therapies have navigated the complicated road to regulatory approval, there are still huge issues with patient access, as a result of the cost of the treatment, limitations in manufacturing capacity, and concerns over safety. Dan Tovar, Senior Director, Head of Cell Therapy ACE

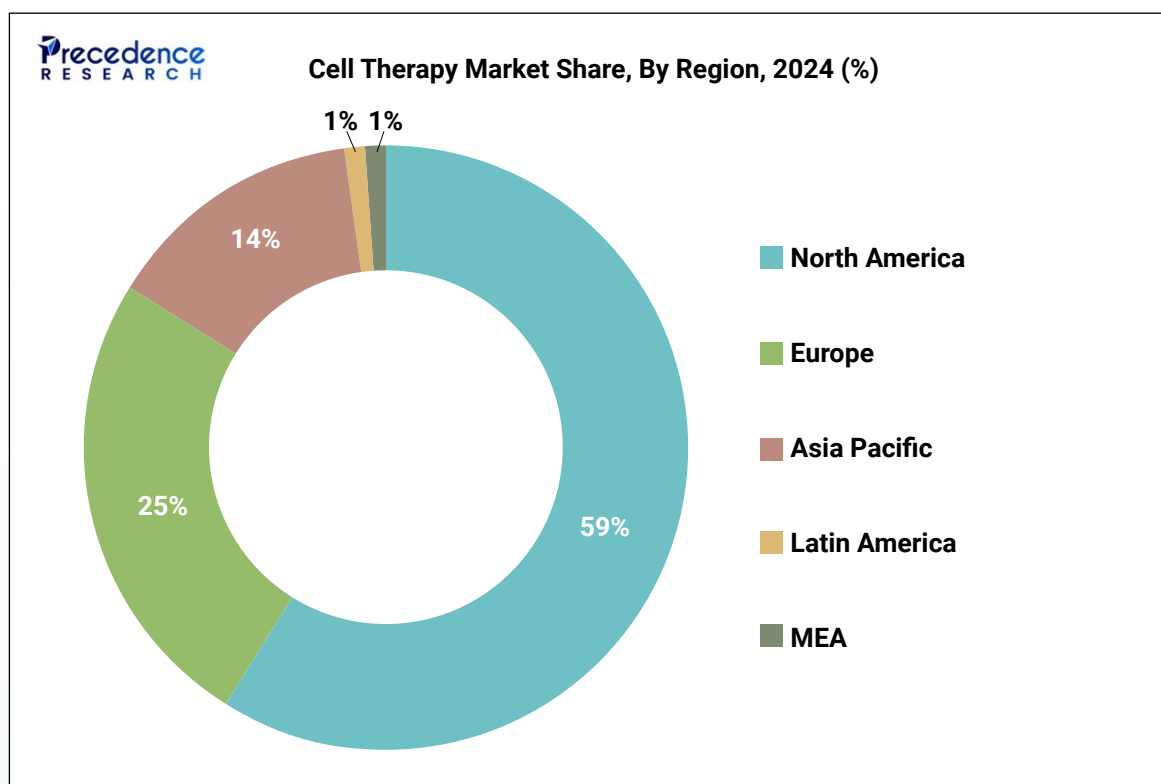
Midsize Markets, Medical Affairs at Kite Pharma, explains the situation in an interview with DDW: "CAR T-cell therapy is one of the most exciting medical breakthroughs to occur in decades, however, despite the therapies being available for nearly seven years, many eligible people living with difficult-to-treat blood cancers are not getting access to these innovative treatments which may change their outcomes and support them living longer lives. In order to deliver CAR T-cell therapy to patients, the entire healthcare ecosystem needs to work together to overcome critical infrastructure challenges – driving healthcare system awareness, investing in healthcare system capacity, improving access to treatment centres and improving knowledge and awareness."

Overcoming barriers to access New evidence reduces safety concerns

Safety concerns in relation to cell therapies, though particularly CAR-T therapies, include the aforementioned cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and the risk of secondary T cell malignancies. In November 2023, the FDA launched an investigation into the safety of BCMA- or CD19-directed autologous CAR-T cell immunotherapies, following reports of T cell malignancies, including chimeric antigen receptor CAR-positive lymphoma. However, in response, the International Society for Cell & Gene Therapy (ISCT) published an expert consortium commentary stating that the benefits of CAR-T therapies continue to outweigh potential risks in the vast majority of cases.

Real-world data has since shown these risks to be very low. A recent study of the French DESCAR-T registry indicates a very low risk of T cell malignancy after CAR T cell therapy⁷. The authors analysed the DESCAR-T registry database, encompassing all paediatric and adult patients with haematologic malignancies who received CAR-T cell therapy in France since 1 July 2018. After a median follow-up of 12.7 months for B cell lymphoma, 17.7 months for B cell ALL and 6.3 months for multiple myeloma, only one (0.03%) of the 3,066 patients included developed a T cell malignancy after CAR-T infusion.

In June 2024, the European Medicines Agency approved a Type II variation to the Summary of Product Characteristics (SmPC) for Kite's CAR-T cell therapies, Yescarta (axicabtagene ciloleucl) and Tecartus (brexucabtagene autoleucl), which included a reduction in daily hospital safety monitoring for patients following CAR-T cell therapy infusion from 10 to seven days. The Agency said the decision to reduce this was supported by further data from clinical trials, post-marketing studies, and updated peer-reviewed publications. "This approval



Source: <https://www.precedenceresearch.com/cell-therapy-market>

is good news for patients, caregivers and physicians, and support capacity in healthcare systems for eligible patients,” comments Dominique Tonelli, Executive Director, Head of Medical Affairs, ACE at Kite. “It reinforces the wealth of clinical and real-world evidence available for axicabtagene ciloleucel and brexucabtagene autoleucel, that supported this reduction in monitoring timelines, and enabling physician discretion in the subsequent management of their patients.”

Tackling infrastructure challenges

The slow nature of the process and a simple lack of manufacturing capacity are other hurdles that are preventing patient access to cell therapies, though many initiatives are currently in place to tackle these. In January 2024, the FDA approved a manufacturing process change that reduced Kite’s median turnaround time (TAT) from 16 days to 14 days. “Given that each cell therapy batch is unique to each patient, manufacturing is central to how we deliver our therapies, and quality, reliability, and speed are critical,” says Chris McDonald, SVP, Global Head of Technical Operations. “As the global leader in cell therapy, patients and physicians count on our 96% manufacturing success rate, and with a reduced manufacturing time in the US, we will continue to expand the reach of Yescarta to even more patients.”

Many companies are seeking collaborations to address these challenges. In one example,

Autolomous and BioCentriq agreed a partnership to digitise and accelerate process development and manufacturing activities using the autoloMATE platform to streamline its process development, with the ultimate aim of accelerating batch delivery, ensuring patients have faster access to cell therapies. Most recently, to address the need for accuracy and reproducibility in cell therapy manufacturing, along with the shortage of workers skilled in the procedure, Astellas and YASKAWA established a joint venture to leverage the dual arm robot ‘Maholo’, developed by YASKAWA’s subsidiary, Robotic Biology Institute.

Another response to this challenge is the number of allogeneic cell therapies currently in development, providing an ‘off-the-shelf’ option. These include Poseida Therapeutics’ CAR-T therapy P-BCMA-ALLO1, which in a recent Phase I trial produced a 91% overall response rate in patients with relapsed/refractory multiple myeloma (RRMM), and Allogene Therapeutics’ ALLO-329, which has been given the green light from the FDA for a rheumatology basket study. Finally, *in vivo* cell therapies are removing this manufacturing time completely. EsoBiotec, recently acquired by AstraZeneca, is one company exploring this option and has launched a clinical trial in China of ESO-T01, its immune shielded lentiviral vector that reprograms T lymphocytes *in vivo* into BCMA CAR-T cells. These innovations will be explored further later in this report.

2024	Developers (Snapshot value)	Clinical Trials (Snapshot value)	Investment (2024 Total)
North America 	1,230	981	\$11.8B
Asia Pacific 	1,029	879	\$1.5B
Europe 	581	384	\$2.0B
Total (y/y growth)	2,936* ↑6%	1,975* ↑3%	\$15.2B* ↑30%

Source: <https://alliancerm.org/wp-content/uploads/2025/01/2025-final-SOTI-briefing-Jan-13.pdf>

Politics casts uncertainty on regional players

Precedence Research estimates that the North America cell therapy market size reached \$3.56 billion in 2024 and will expand at a CAGR of 23.07% during the forecast period¹. This is currently the dominant global market, with the largest share of 59% in 2024. To further boost the market in the US, in December 2024, the Biden-Harris Administration introduced the Center for Medicare & Medicaid Services (CMS) Cell and Gene Therapy Access Model, with the aim to improve health outcomes, increase access to cell and gene therapies, and lower health care costs. Gene therapies Lyfgenia and Casgevy have been the first to enter the scheme, though it is hoped with continued support from the new Trump administration, more manufacturers will follow suit.

In contrast, planned cuts to medical research funding from the National Institutes of Health (NIH), along with the expected brain drain as US researchers move to more supportive countries, could represent a potential threat to this global dominance. In a statement, the American Society of Cell & Gene Therapy (ASCGT) said: "The United States is the clear world leader in cell and gene therapies. In 2024, the pipeline of gene, cell, and RNA therapies had 4,238 therapies in development. Consistent funding over many years from the NIH has been critical for these advances, providing one of the most efficient returns on investment of federal dollars. Cuts to NIH-covered indirect costs would devastate the infrastructure and support that is critical for research at universities and institutions across the United States." At the time of writing, the NIH cuts remain blocked by a nationwide injunction issued on 5 March 2025, with the Trump administration expected to appeal.

Unfortunately, a lack of consistency across Europe

is limiting the region's potential in developing and leveraging advanced therapies. According to the European Federation of Pharmaceutical Industries and Associations (EFPIA), advanced therapy medicine products (ATMP) clinical trial activity was twice as high in the US and almost three times as high in China than in Europe⁶. They report that the number of ATMP trials conducted in the US and Asia-Pacific region grew by 70% and 67% respectively from 2014-21, while Europe remained stagnant. They also report that the US currently has 50% of the world's ATMP manufacturing facilities, stating that "Asia is fast becoming the most competitive region for attracting ATMP clinical trials (255 in 2021), but Europe is unfortunately in decline (89 in 2021)"⁶. Although the European Medicines Agency (EMA) is taking positive steps toward simplifying the regulatory process at a European level with the clinical trial regulation (CTA) adopted in 2022, there is still the risk that approved therapies may not reach patients in all EU countries due to delays in local availability and the lack of appropriate legislation.

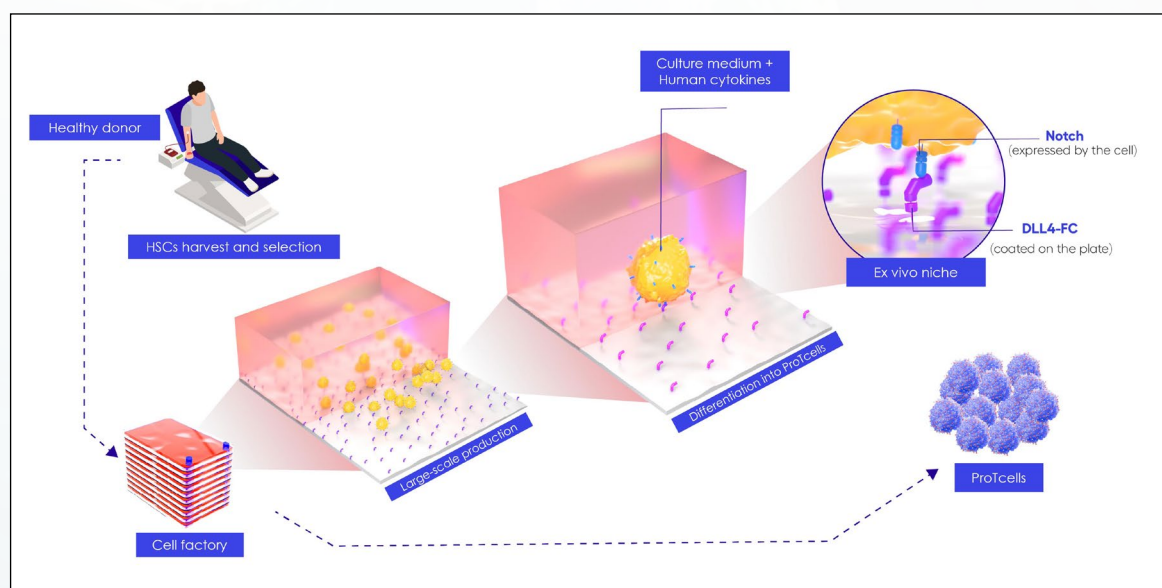
In light of these headwinds against the dominant markets, Precedence Research also expects Asia-Pacific to develop at the fastest growth rate of 16% to 2030, dominated by China and due to the favourable government regulations in this region.

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A new T cell force: Harnessing the thymus to restore the immune system

Olivier Negre PhD, Chief Scientific Officer at Smart Immune, explores a novel approach to overcoming the challenges of T cell therapy.



It's well known that you can't live well, or for very long, without T cells. And when T cells don't function normally, problems arise. T cell dysfunction is at the root of a wide range of diseases. These white blood cells are the key to adaptive immunity and defend us against viruses, fungi, certain bacteria, and tumours.

T cell dysfunction-related diseases such as inherited (e.g. mutations in genes on the X chromosome) or acquired (e.g. drugs, radiations, infections like HIV) immunodeficiencies affect a large proportion of the global population. Sometimes, the most advanced therapies' efficacy is hampered by a poor T cell count or quality, as seen in treatments based on T cell engagers and chimeric antigen receptor (CAR) T cells. Consequences of T cell dysfunction thus represent a massive and overlooked medical need.

T cell therapy: The challenges

T cell therapies are booming and are expected to represent \$85 billion in 2030. However, the field still faces several challenges, including cell exhaustion,

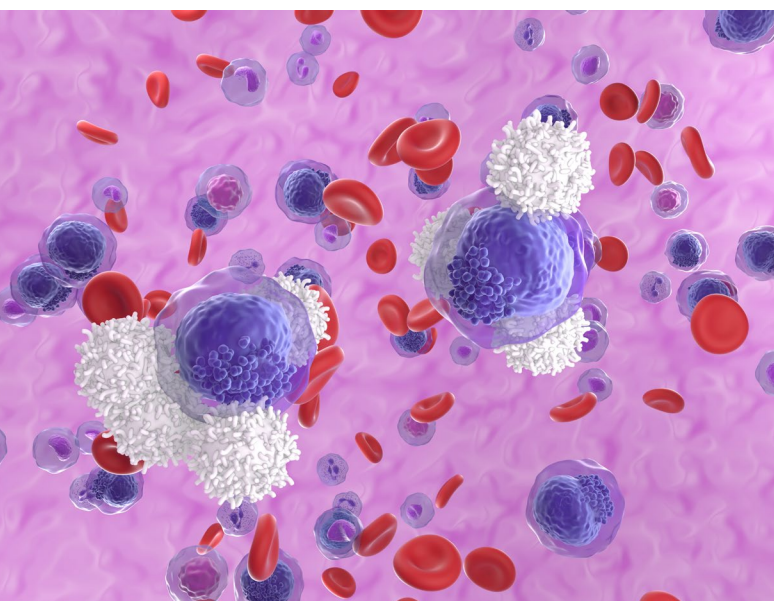
toxicity, complex manufacturing, rejection and scalability.

The T cell-progenitor approach aims to address these pitfalls. By generating T cell progenitors from healthy donors and harnessing the patient's thymus, Smart Immune hopes to offer a well-tolerated, long-lasting and safe treatment.

The goal is to create biobanks of off-the-shelf products that are rapidly available to patients at a lower cost per dose. Cells in Smart Immune's drug candidate SMART101 are not edited and keep their T cell receptor, allowing the restoration of a full polyclonal T cell force for the patients that will hopefully see long-lasting benefits.

Combining technology and physiology

Interestingly, T cell progenitors had never been exploited before, as they are found in too small quantities in the blood. Smart Immune's Isabelle André, PhD and Marina Cavazzana, MD, PhD have developed a process and a cocktail of cytokines that



enable blood stem cells (CD34) to be differentiated into progenitors, whether they are derived from mobilised peripheral blood, used in the production of clinical batches, or from cord blood.

The feeder-cell-free *ex vivo* cell culture system can differentiate blood stem cells into T cell progenitors in a week, thanks to a fusion protein that can strongly activate the Notch pathway.

In this approach, it is the thymus that 'tolerises' and personalises the treatment. Indeed, once immature T cells are infused into patients, their thymus selects about 1% of the best, self-tolerant cells and trains them to become fresh, diverse, and fit T cells. The thymus also multiplies ProTcell-derived T-cell populations by a factor of around 1,000.

Clinical data and potential applications

Smart Immune's cell therapy SMART101 (allogeneic blood stem cell-derived T cell progenitors) is being studied in an international multicentre trial called ReSET-02, in adults with high-risk acute leukaemia who have received a partially compatible (haploidentical) stem cell transplant.

Phase I dose escalation results were promising and were presented at the ASH Congress last December. Encouragingly, the investigators have been able to define the recommended dose at its highest tested level. SMART101's safety profile is excellent, and early signs of biological efficacy were observed, which remain to be confirmed with further enrolments in randomised Phase II clinical trials, but there are grounds for optimism.

Additionally, initial data showed higher T cell counts within two to three months, compared with 12-

18 months needed in the standard of care. This timesaving can be a game-changer for patients. A sufficient CD4 count should enable transplanted patients to receive fewer drugs, taking them out of the 'low T cell danger zone' that exposes them to infections and relapses.

In immuno-oncology, which will be the company's main focus in the medium term, treatments such as T-cell engagers or immune checkpoint inhibitors require a sufficient level of T lymphocytes to be effective. This is why Smart Immune will be testing these T cell progenitors as soon as possible in association with these new therapies in solid tumour indications.

The preclinical team is already interested in other potential applications, such as CAR ProTcells, which could add to all the benefits of ProTcell's ability to target a specific type of cancer or autoimmune disease; and are also considering applications to fight AIDS, with HIV-resistant ProTcells, and applications to combat immune ageing. Preclinical models have already shown that the progenitors repopulate, reinvigorate, and awaken aged and/or irradiated thymuses.

Summary

T cell dysfunction stands at the core of numerous devastating diseases, creating an urgent but often overlooked medical need affecting millions globally. These essential white blood cells, responsible for our adaptive immunity, protect us against viruses, fungi, certain bacteria, and cancerous cells—yet when they malfunction, our entire immune system becomes compromised. Even the most advanced therapeutic approaches are frequently hampered by insufficient T cell counts or poor-quality T cells, leaving patients vulnerable to infections and disease progression. New approaches like T cell-progenitor therapy have the potential to address this critical gap by rapidly restoring a robust pool of healthy, functional T cells in patients with compromised immune systems.



About the author

Olivier Negre PhD is the CSO of Smart Immune with over 20 years of industry experience and helped develop the first approved gene therapy for beta-thalassemia. He is a board member of the French Society of Gene and Cell Therapy, co-president of Gene & Cell Therapy Institute and co-founder of Biotherapy Partners.

Machine learning and cell-free expression: Shining a light on the dark matter of proteins

DDW's **Diana Spencer** speaks to **Matt Deaner**, Staff Scientist at Elegen, about the future of protein engineering and how the company can support AI workflows to advance drug discovery.

DS: What are the greatest challenges faced by researchers when trying to find the optimal protein sequence?

MD: Protein sequences evolved over billions of years—but engineering even a small protein, like a 100 amino acid antibody, is an enormous challenge: there are 20¹⁰⁰ possible DNA sequence designs. That's an astronomical space to search for the optimal design! Also, even with a known natural sequence, small mutations can have unpredictable effects on the folding or the catalytic activity of that protein sequence.

While this complexity is understood, it highlights two major bottlenecks in protein optimisation: first, the difficulty of acquiring DNA to build individual protein variants; and second, the challenge of screening them downstream, especially for catalytic activity, which often requires analysing one variant at a time with techniques like HPLC. These constraints make both design and testing significant hurdles in the protein engineering process.

DS: What are the limitations of common methods of protein engineering?

MD: Because of the complexities of protein engineering, most efforts focus on very small regions of a protein and limited combination of mutations. A common approach is site saturation mutagenesis, where researchers target specific residues believed to impact function, mutate them to all 19 amino acids, and evaluate protein performance. They then combine the best performing mutations from each site, hoping the result is the most optimised protein.

A major challenge in protein engineering is epistasis, where combinations of mutations that perhaps didn't perform well when screened individually can have an optimal effect when combined together. A mutation that seems ineffective on its own might enhance

function when paired with others, depending on how the protein binds ligands or catalyses a reaction. Traditional methods like site saturation mutagenesis optimise residues individually and then combine the best. But this approach misses the complex interactions between mutations. Without the ability to build and test all possible combinations within an active site, we're likely overlooking more optimal protein designs.

DS: How can machine learning-based approaches, like Active Learning-assisted Directed Evolution (ALDE), overcome these challenges and increase accuracy?

MD: Machine learning methods are highly valuable because they can use more complex representations of a sequence than just amino acid primary sequence. The ALDE method also takes into consideration other factors such as evolutionary conservation of amino acids.

Machine learning models can be trained on a relatively small set of mutations (<96). Focusing on important residues within the protein, they can predict optimal combinations that would otherwise be missed by an iterative optimisation of each individual residue. Since the amount of data required to train AI models for prediction is experimentally manageable, it allows researchers to more quickly find optimal combinations of mutations and skip over the laborious process of directed evolution.

DS: How have these approaches impacted drug discovery in practice? Can you give some examples?

MD: A recent example is from the Jewett lab at Stanford. They took a native enzyme, the mcbA enzyme from *Marinactospora thermotolerans*, which is known to have promiscuous activity to form amide bonds. They found that just by changing out the carboxylic acid and amine substrates and testing <96 single mutation mcbA variants, they

could identify optimal combinations of mutations to produce a given target amide. The model could predict combinations of mutations from within a possible set of hundreds of thousands that would be optimal for producing any one of these amide molecules. In addition, the researchers also leveraged a cell-free protein expression (CFPS) system that allowed them to quickly express the proteins and characterise performance with HPLC, avoiding living cells entirely. This allowed them to go from target amide to optimised variant within the span of about a week. Since the mcbA enzyme is quite promiscuous for diverse amines and carboxylic acids, there's a huge diversity of molecules that can be quickly produced just by feeding in different substrates and testing a 96-well plate of protein variants.

In drug discovery, synthetic chemistry is widely used, but enzymes offer superior stereo-selectivity—with the ability to produce precise molecular structures synthetic chemistry can't easily achieve. It's now possible to quickly engineer enzyme variants that can produce target molecules for screening, making protein engineering a powerful complement to synthetic chemistry in building compound.

DS: Artificial proteins have been created using AI tools. What are protein large language models (LLMs), and what impact could they have?

MD: Protein large language models (LLMs) are trained on natural proteins sequences to learn the 'grammar' of how amino acids interact, enabling them to design entirely new, functional proteins. A recent breakthrough from Stanford used an LLM to predict the 3D structure of the SARS-Cov-2 antibody from its sequence, leading to a redesigned variant with a 25-fold better binding affinity than the FDA-approved treatment for the variant in 2022. This LLM approach outperformed traditional methods like directed evolution and screening.

DS: Are there any downsides to machine learning in this context? Is there anything researchers need to be aware of?

MD: The main challenge with machine learning models is bias in the training data and results that can be difficult to interpret. They also still produce many misses, so researchers must test dozens to potentially hundreds of variants to find the most optimal protein.

DS: What services does Elegen provide to optimise these workflows?

MD: Traditional protein engineering methods like directed evolution rely on generating large libraries.

For example, NNK libraries used in for site saturation mutagenesis generate millions of protein variants in a single tube—but researchers often screen just a small fraction of these, selected at random and without control over which variants are tested. In contrast, machine learning models like the ALDE model from Francis Arnold's lab recommend a defined set of 96 protein variants to train the model with the most informative data. The challenge now is synthesising these targeted variants quickly and in parallel.

That's where Elegen comes in. We can synthesise these variants up to 7kb with clonal accuracy and a turnaround time of just seven business days—and at a lower cost than plasmid DNA at most lengths. With this rapid synthesis, researchers could iteratively test 96 variants, retrain their model, and then order another 96 variants, continuously training on and refining with each cycle.

DS: How do you see AI advancing protein engineering in the next five to ten years?

MD: I don't want to look too speculative, but what excites me most is the potential for entirely novel protein designs. Until now, protein engineering has mostly focused on tweaking natural sequences, but there's a vast space of functional proteins that don't exist in nature and haven't yet been explored. I believe we're on the verge of a big expansion in types of sequences researchers pursue, leading to new protein functions in the next five to ten years. Elegen is uniquely positioned to support this shift. Synthesising complex, non-natural DNA is challenging, but the ENFINIA cell-free platform is robust and built to handle that complexity—quickly and accurately.



Matt Deaner is a Staff Scientist at Elegen working to develop new products based on Elegen's proprietary cell-free clonal synthesis technology. He is an expert in high-throughput molecular biology workflows to accelerate life science R&D, with 5+ years of experience in product development from conception to commercialisation.

The future for cell therapy: New indications and new modalities

Despite the optimism around cell therapies, many are expensive and complicated to manufacture and come with potentially severe side effects. In this article we take a look at the new generation of therapies and how drug developers are overcoming these hurdles to bring cell therapies to more patients than ever.

The next generation of CAR-T therapies

Advancing CAR-T in haematology

While haematological malignancies are a key therapy area for CAR-T therapies, with several regulatory approvals, there is still room for improvement and areas of unmet need. For example, B-cell lymphoma can be successfully treated with CAR-T therapies, yet T-cell lymphoma's still have limited treatments and a low survival rate. March Biosciences' MB-105 is a first-in-class CD5-targeted CAR-T cell therapy for patients with relapsed or refractory CD5-positive T-cell lymphoma. Since the start of 2025, the company has received orphan drug designation from the US Food and Drug Administration (FDA) and launched a Phase II trial. Sarah Hein, Co-Founder and Chief Executive Officer, comments: "CAR-T therapies have revolutionised the treatment of B-cell lymphomas and leukaemias but have not successfully addressed the rarer T-cell lymphomas

and leukaemias. We are optimistic that this larger trial will further validate MB-105's potential to address the critical unmet needs of these patients and look forward to reporting our first clinical readouts."

SyntheKine is another company hoping to improve CAR-T outcomes, this time in relapsed or refractory CD19+ haematologic malignancies. Its unique approach combines an autologous CD19-targeting CAR-T cell which expresses an engineered IL-2 receptor (SYNCAR-001) with an engineered pegylated IL-2 cytokine that selectively signals through the engineered IL-2 receptor (STK-009) to improve the therapeutic effect and reduce toxicity. In addition to blood cancers treatment, the FDA has granted fast track designation to SYNCAR-001 + STK-009 for patients with severe, refractory systemic lupus erythematosus (SLE) – an emerging market for CAR-T therapies.

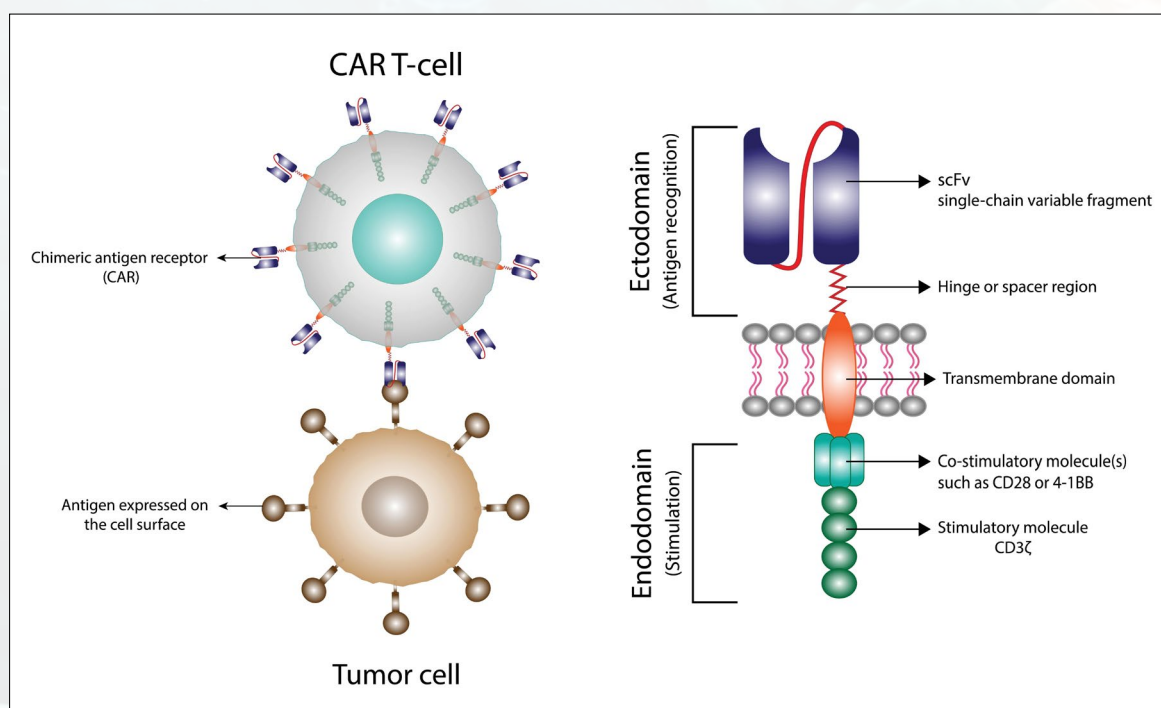


Figure 1: The science behind CAR-T therapy.

Dr Lia Palomba, Memorial Sloan Kettering Cancer Center, explains the science behind the combination: “Enhancing CAR-T cells with consistent cytokine support, particularly IL-2, has the potential to significantly improve their therapeutic effect. However, prolonged wild-type IL-2 administration is currently not feasible due to severe toxicities like capillary leak syndrome (CLS). SYNCAR-001 + STK-009 represents an innovative solution, enabling the targeted delivery of a potent and durable IL-2 signal specifically to CAR-T cells *in vivo* and avoiding activation and proliferation of native lymphocytes that can cause CLS.”

A different approach to reducing toxicity is to reduce the amount of time the CARs engage with their target, in theory causing less inflammation and allowing the CAR-T cells to persist for longer in patients. In the FELIX clinical trial, led by researchers at University College London (UCL) and UCLH and sponsored by UCL spin-out Autolus Therapeutics, obecabtagene autoleucel (obe-cel) produced remission in 74.7% of patients with relapsed/refractory B-cell acute lymphoblastic leukaemia (r/r B-ALL).

CAR-T for solid tumours

CAR-T therapies have so far had limited success in solid tumours and many companies are keen to explore this untapped potential. A team of investigators from City of Hope in the US recently reported promising therapeutic activity in a first-in-human Phase I clinical trial of a CAR-T therapy for prostate cancer. The CAR-T cells targeted prostate stem cell antigen (PSCA), which is highly expressed in prostate cancer. In another development, in 2024 the FDA approved a Phase II clinical trial for Biosynge’s BRG01, an EBV-specific CAR-T cell therapy, for the treatment of relapsed/metastatic EBV-positive nasopharyngeal carcinoma.

Taking on autoimmune diseases

A number of companies are pursuing CAR-T therapies in autoimmune diseases alongside SyntheKine, and these have so far shown promise in potentially help patients achieve drug-free remission. Vinie Varkey, Healthcare Analyst at GlobalData, comments: “The objective of CAR T-cell assets in patients with autoimmune diseases is to achieve drug-free remission, which itself represents as a very high target to meet for any treatment modality. Given the promise of CAR T-cells in oncology, there is a strong belief in this strategy, along with a concerted action from industry and academic players to achieve similar results in autoimmune indications.”

Kyverna is leading the way in this market. The company’s CAR-T therapy KYV-101 has so far shown potential in several B cell-driven autoimmune

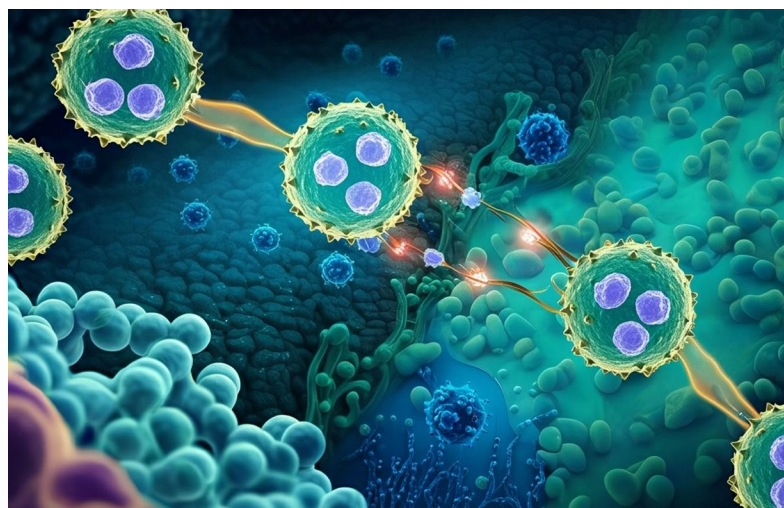


Figure 2: CAR-T therapy in lupus.

diseases including lupus nephritis (LN), myasthenia gravis (MG), multiple sclerosis (MS), and stiff-person syndrome (SPS). Following positive initial results, the company has received approval from the FDA for a Phase II trial in its ongoing KYSA-8 programme in SPS, which has enrolled 70% of study participants, completed patient enrolment in KYSA-6, its Phase II trial in MG, and is advancing two Phase I/II trials in LN, KYSA-1 and KYSA-3.

Professor Georg Schett, from the Friedrich-Alexander-University in Erlangen, Germany, comments on the LN data so far: “The data reinforce that treatment with KYV-101 drives deep B cell depletion in patients with LN, and appears to reset the immune system, stabilise eGFR, preserve kidney function and enable clinical improvement in SLE activity. Notably, this clinical benefit is occurring while also eliminating immunosuppressants and reducing glucocorticoids to physiologic levels with a manageable safety profile.”

Are *in vivo* CAR-T therapies the future?

One way to address many of the challenges with current CAR-T therapies is to reprogramme immune cells directly in the body, which could simplify the manufacturing process, shorten the delay to treatment, and bypass the need for lymphodepletion chemotherapy, however, these are still very expensive and raise new safety concerns.

EsoBiotec and partners Pregene Biopharma are investigating ESO-T01, an immune shielded lentiviral vector that reprogrammes T lymphocytes *in vivo* into BCMA CAR-T cells, for relapsed/refractory multiple myeloma. The company’s initial clinical observations indicate a favourable safety profile and promising efficacy, with pharmacokinetic characteristics comparable to autologous *ex vivo* CAR-T therapies. “ESO-T01 has the potential to offer a simplified patient journey, with several benefits over current

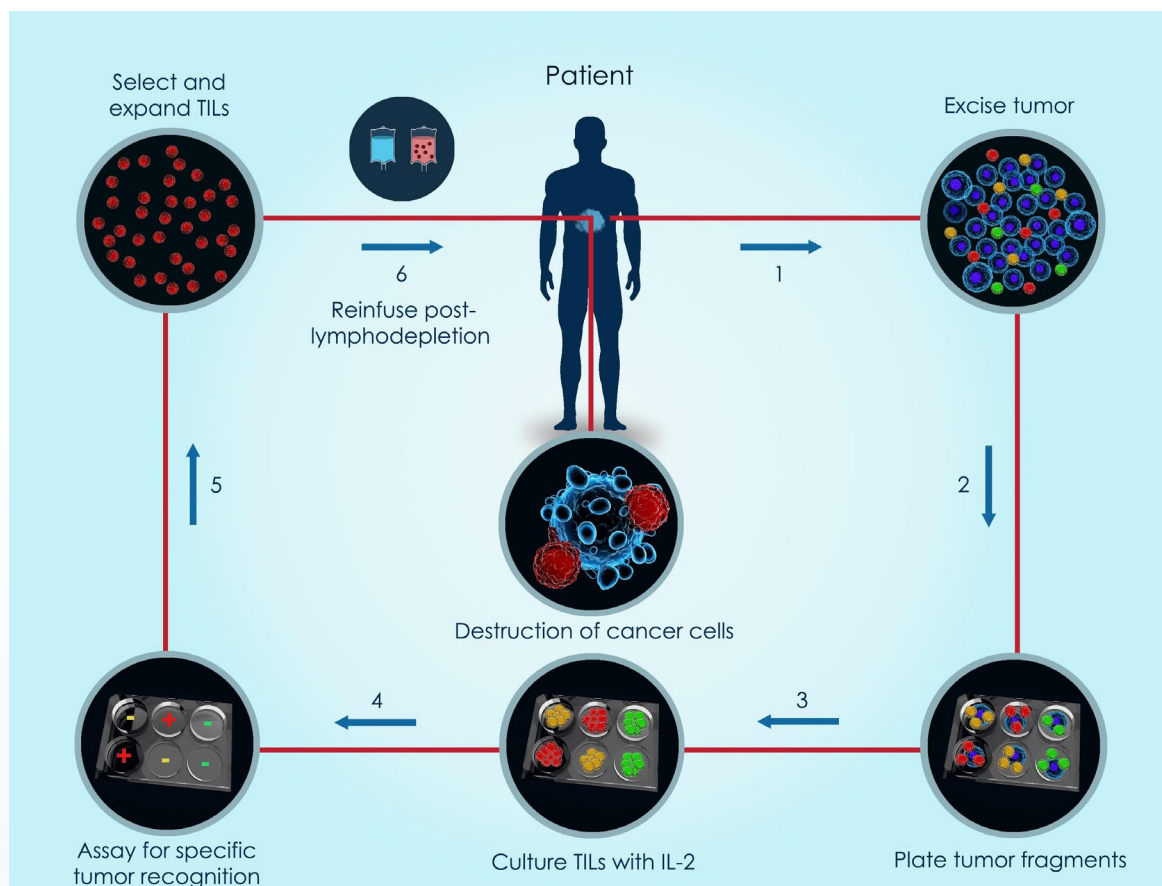


Figure 3: The use of tumour-infiltrating lymphocytes (TILs) for the treatment of cancer.

treatments for multiple myeloma that are often costly and have unfavorable side effects,” says EsoBiotec CEO Jean-Pierre Latere. “Our treatment does not require lymphodepletion, is immediately available to patients without any waiting time, and is administered in a single intravenous dose that takes less than 10 minutes.”

Capstan Therapeutics, on the other hand, is advancing *in vivo* reprogramming of cells through RNA delivery using targeted lipid nanoparticles (tLNP) for autoimmune diseases. Its lead *in vivo* CAR-T candidate CPTX2309 delivers an mRNA payload encoding an anti-CD19 CAR to preferentially reprogramme CD8-expressing cytotoxic T cells. The preclinical data has shown robust and preferential engineering of functional CD8+ CAR T cells *in vitro* and in small and large animal models, with up to 80% of CD8+ T cells reprogrammed to express CAR in non-human primate models. Most importantly, these results are consistent with what has been observed in autoimmune patients receiving conventional *ex vivo* engineered CD19 CAR-T therapy.

Off the shelf CAR-T

Another approach to make CAR-T more accessible is by creating off-the-shelf allogeneic therapies that could reduce costs by eliminating the need for

apheresis, reduce logistics, and increase production scalability.

Allogene Therapeutics is one company taking the lead in this area. Last year the company announced the initiation of a Phase II trial evaluating cemacabtagene ansegedleucel (cema-cel) as part of the first line treatment regimen for newly diagnosed large B-cell lymphoma (LBCL) patients who are likely to relapse. According to David Chang, President, Chief Executive Officer and Co-Founder, the product is “designed to eliminate the complex logistics that have hindered autologous CAR-T adoption to date and open the door for access by doctors in the community setting”. In February 2025, Allogene was given FDA approval for a Phase I basket trial to evaluate ALLO-329 in patients with systemic lupus erythematosus, including lupus nephritis, idiopathic inflammatory myopathies, and systemic sclerosis. ALLO-329 features a dual-targeting design against CD19+ B-cells and CD70+ activated T-cells, aiming to address both B-cell and T-cell dysfunction. If the trial is successful, Allogene says it has the capacity to manufacture upwards of 60,000 doses per year, and expand access to CAR-T therapy across a wide range of autoimmune disease indications.

In late 2024, investigators saw a 91% overall

response rate in patients with relapsed/refractory multiple myeloma (RRMM) in a Phase I trial of allogeneic CAR-T therapy P-BCMA-ALLO1 and lymphodepletion. P-BCMA-ALLO1 is an investigational non-viral, stem cell memory T cell (TSCM)-rich allogeneic CAR-T cell therapy being developed by Poseida Therapeutics and Roche, which has been granted Regenerative Medicine Advanced Therapy (RMAT) designation from the FDA. "The high overall response rate of 91% is remarkable because most study participants in my centre had rapidly proliferative refractory disease," comments Bhagirathbhai Dholaria, Associate Professor of Medicine, Malignant Hematology & Stem Cell Transplantation at Vanderbilt University Medical Center in Nashville. "All patients in the Phase I trial have been treated quickly once enrolled, with no waiting for manufacturing, with no need for apheresis or bridging therapy, demonstrating key advantages of allogeneic CAR-T cell therapy."

Beyond CAR-T – other cell therapies in development

It is usually CAR-T therapies that make the headlines but they are just one type of adoptive cell therapy, others including tumour-infiltrating lymphocyte (TIL) therapy, engineered T cell receptor (TCR) therapy, regulatory T cell (Tregs) therapy, and natural killer (NK) cell therapy. The TIL approach harvests naturally occurring T cells that have already infiltrated patients' tumours, activates and expands them and then re-infuses them back into patients. TCR therapy requires taking T cells from patients and equipping them with a new T cell receptor that enables them to target specific cancer antigens. Tregs are a subset of CD4+ T cells that have anti-inflammatory properties.

Several companies have had early success with natural killer (NK) cell therapy. One of these is NKGen Biotech, which, in September 2024, announced promising early interim data from its Phase I cohort trial of troculeucel, a cryopreserved autologous enhanced NK cell therapy for moderate Alzheimer's disease (AD). After three months with a dose of six billion cells per treatment, the three patients in the Phase I cohort had no drug-related adverse reactions and two of the patients went from a moderate to a mild AD rating on the Clinical Dementia Rating-Sum of Boxes scale. The company has since earned Fast Track Designation for this treatment from the FDA.

Chinese company Grit Biotechnology is pursuing TIL therapies in advanced solid cancer patients and has received approval to launch clinical trials from both the FDA and China Center for Drug Evaluation (CDE). According to the company, GT201 surpasses traditional TIL therapies in proliferation, tumour-killing efficacy and long-term survival with reduced

dependence on IL-2. Its non-gene-engineered TIL programme, GT101, is currently in Phase II trials and is on track to file for Biologics License Application (BLA) in 2025, making it the leading TIL pipeline in China.

Another company active in the TIL market is Iovance. The company's Amtagvi (lifileucel) became the first cellular therapy indicated for the treatment of metastatic melanoma as well as the first FDA-approved tumour-derived T cell immunotherapy in February 2024 when it was approved through the Accelerated Approval pathway. Steven Rosenberg, Chief, Surgery Branch, National Cancer Institute, comments on the approval: "This landmark FDA approval reflects significant advancements in TIL cell therapy since we initially showed that TIL cells isolated from patients with metastatic melanoma could be expanded in the lab and returned to the patient to mediate cancer regression. This approval is transformative for the entire research field and supports continued investigation of TIL cell therapy across additional types of solid tumours."

TCR therapy is showing promise as a "universal" cancer treatment in a number of studies. The first TCR therapy to be approved by the FDA was Adaptimmune's afamitresgene autoleucel, or afamicelel (Tecelra), to treat metastatic synovial sarcoma. Meanwhile, SCG Cell Therapy is using the approach to treat hepatitis B virus, using an autologous hepatitis B virus (HBV)-specific T-cell receptor-engineered T cell (TCR-T) therapy targeting hepatitis B surface antigen (HBsAg). In a Phase I clinical trial, SCG101 exhibited promising antiviral activity in patients with advanced HBV-related hepatocellular carcinoma (HBV-HCC).

PolTREG is one company focusing on Treg therapy, and by combining these with other cell therapies, the company is currently advancing a pipeline of polyclonal TREG, CAR TREG, allogeneic TREG, antigen-specific TREG, and TCR TREG therapies in a range of autoimmune indications. The company launched a placebo-controlled Phase II clinical trial with PTG-007 Treg cell therapy in presymptomatic type-1 diabetes (T1D) patients in late 2024. The therapy has shown promise in other indications. In an exploratory Phase I/IIa safety study in relapsing-remitting multiple sclerosis, patients who had received intrathecal administration of PTG-007 showed no increase in existing lesions in the brain, and almost no new lesions, a far superior result compared to those who had received the therapy intravenously. Most recently, PolTREG received a non-refundable grant of \$1.7 million from the Polish Medical Research Agency to carry out preclinical studies of its antigen-specific regulatory Treg project by the end of Q1 2026.

Machine Learning and Cell-Free Expression:

Shining a Light on the Dark Matter of Proteins

DDW's Diana Spencer speaks to Matt Deaner, Staff Scientist, Product Development, Elegen, about the future of protein engineering and how the company can support AI workflows to advance drug discovery.

Topics Covered

- The greatest challenges faced when trying to find the optimal protein sequence
- Limitations of common methods of protein engineering
- How machine learning-based approaches overcome these challenges and increase accuracy
- How artificial proteins have been created using AI tools
- Predictions on how AI advancing protein engineering will advance in the next next five to ten years



Matt Deaner is a Staff Scientist at Elegen, a leader in next-generation DNA manufacturing. He thrives on solving complex problems, often experiencing “a-ha moments” that lead to significant breakthroughs. Notably, his innovative troubleshooting was pivotal in the launch of Elegen’s flagship product, ENFINIA DNA.