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Improving CAR-T cell therapy, step-by-step

1 Plain language summary

Chimeric antigen receptor (CAR)-T cell therapy is an innovative cancer treatment that uses a patient's immune system to combat tumour cells. A patient's T cells are genetically modified, which allows them to recognize and bind to a specific target antigen on cancer cells. CAR-T cell therapy is a very effective therapy in treating different types of cancer, however, the production process is long and complicated, with only a few specialized centres able to manufacture CAR-T cells.

This review explores the bottlenecks in every step of the CAR-T cell production process, focusing on T cell isolation, genetic modification, T cell expansion, and good manufacturing practice (GMP) regulations. The first identified bottleneck is the use of a patient's own material, this causes a long wait time since production starts after isolation from the patient's T cells. A possible solution is using T cells from a donor to create CAR-T cell therapy, so that is ready to use when a patient needs it. The identified bottleneck with genetic modification is the use of viral particles, which has safety concerns and is therefore highly regulated. There is currently a lot of research being done to bypass the use of viral particles, such as different ways of gene editing. Furthermore, the bottleneck in T cell expansion is that it takes 9-14 days to expand the cells after genetic modification. Research shows that this is not necessary and it could even be beneficial for the effectiveness if it was shortened. Finally, GMP regulations limit the availability of CAR-T cell therapy since only a few centres are able to comply with these regulations.

This review also explores completely new strategies for CAR-T cell production, such as *in vivo* CAR-T cell production, which involves engineering T cells inside the patient's body, potentially reducing the time and complexity of the manufacturing process.

After comparing all the different solutions and strategies, the *in vivo* CAR-T cell production and the use of donor material are the most promising solutions for optimizing the production process of CAR-T cell therapy. This would be even better if hospitals could locally produce CAR-T cell therapy to improve the availability.

In conclusion, while CAR-T cell therapy has shown great promise in cancer treatment, the production process is complex and requires further optimization to make it more efficient and accessible to more patients.

Content

2 Introduction

CAR-T cell therapy is a cancer immunotherapy that harnesses a patient's own immune system to fight cancer. A patient's T cells are engineered to express a chimeric antigen receptor (CAR). A CAR is a synthetic receptor that is made by combining the antigen-binding domain of an antibody with the signalling domain of a T cell receptor. This allows the T cells to recognize and bind to a specific target antigen on cancer cells, even when they have lost their natural ability to do so (Sterner & Sterner, 2021). This innovative therapy is able to effectively treat haematological malignancies with seven therapies approved by the U.S. Food and Drug Administration (FDA) (Chen et al., 2022).

While CAR-T cell therapy is highly efficient, the production process is long and complicated (Figure 1). First, the T cells are collected from the patient via leukapheresis and shipped to a manufacturing facility. There, the T cells need to be activated after which they are genetically modified to express the CAR (Agarwalla et al., 2022). Genetic modification is achieved by lentiviral vectors which reverse-transcribe the RNA permanently into the DNA of T cells (Zhang et al., 2017). CAR-T cells are then expanded for 9 to 14 days until the cells are ready for infusion (Ghassemi et al., 2018). The production process needs to be performed under good manufacturing practices (GMP) to ensure the quality of the final cell product (Blache et al., 2022).

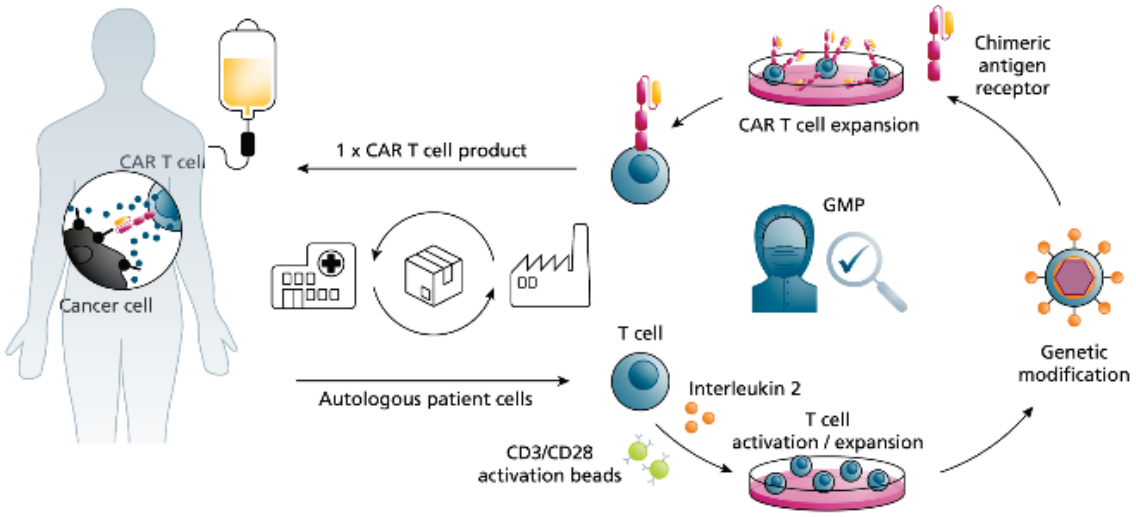

Figure 1: CAR-T cell production process (Blache et al., 2022)

The lengthy and labour-intensive production process is a big limitation of CAR-T cell therapy, with only few specialised centra able to manufacture CAR-T cells (Agarwalla et al., 2022). This review explores the bottlenecks in the production process, focussing on T cell isolation, T cell activation, genetic modification, expansion, and GMP regulations. Experts in the field of cell therapy and GMP have been consulted to highlight the bottlenecks they experience on a daily basis. Possible solutions and new strategies are explored based on the identified bottlenecks.

3 T cell isolation from the patient

3.1 Background

Autologous material is used for the production of CAR-T cells, therefore the T cells need to be isolated from the patient via leukapheresis. This procedure isolates white blood cells through centrifugal force while returning the other blood components into the bloodstream of the patient (Depil et al., 2020). The main reason for autologous material is to warrant the safety of the therapy since donor material could result in graft-versus-host disease (GvHD) (Larson & Maus, 2021).

3.2 Bottlenecks

While autologous material is the safest choice of material, it causes a need for individual manufacturing where the quality of the cell product is dependent on the patient's material. Following several rounds of chemotherapy, patients often suffer from lymphocytopenia, where they have limited white blood cells. Consequently, their T cells could be insufficient for the production of CAR-T cells. The functionality of the T cells could be diminished by the chemotherapy as well, possibly leading to manufacturing failure. Moreover, patients with rapidly progressive malignancies do not have the time to wait the few weeks it takes to manufacture autologous CAR-T cell therapy (Lin et al., 2021). Limited manufacturing centres around the world are able to produce autologous CAR-T cells which adds to the wait time of patients that would be better helped with instant available therapies (Blache et al., 2022).

3.2.1 Expert opinion

Experts in the field of CAR-T cell production stated that a complicated bottleneck in using autologous material is that the patient material is of lesser quality than the healthy donor material they use to set up the protocols for CAR-T cell production, leading to variable results. Furthermore, they are concerned with the impact the long wait time has on the survival chances of patients with aggressive forms of cancer.

3.3 Possible solutions

Allogeneic CAR-T cell therapy could be the solution for the above-mentioned bottlenecks. The use of healthy donor material would improve the quality and cell yield of the source material, so that a patient's survival chances are not dependent on the quality of their T cells. Additionally, allogeneic CAR-T cell therapy can be made readily available, greatly shortening the wait time. Other advantages are the ability to combine CAR-T cells to target multiple markers of the malignancy and redosing when necessary. Finally, allogeneic CAR-T cell therapy will be easier to upscale and industrialize when multiple doses can be made from one healthy donor (Depil et al., 2020).

However, there is a reason why allogeneic CAR-T cell therapy is not yet used in the clinic, namely the risk of GvHD, which initiates a need for immunogenicity mitigation strategies. A human leukocyte antigen (HLA) mismatch between the patient and the donor CAR-T cell could induce GvHD, causing the patient tissue to be damaged by the CAR-T cells. The T cell receptor (TCR) on the allogeneic CAR-T cell recognizes the unknown HLA of the patient, causing an adverse reaction. Consequently, eliminating TCRs from the CAR-T cells through gene editing could reduce the chances of GvHD (Lv et al., 2023). One example is a study by Eyquem et al. (2017) which was able to insert the CAR gene into the TCR gene using clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9 (CRISPR-Cas9). The CAR-T cells successfully expressed CAR while not expressing TCR, showing that allogeneic CAR-T cell therapy without the risk of GvHD is possible.

Another strategy would be the use of natural killer (NK) cells instead of T cells since NK cells do not express alloreactive receptors. Liu et al. (2020) studied the safety and efficacy of CAR-NK cell therapy in clinical trials, using HLA-mismatched CAR-NK cells. The efficacy of CAR-NK cell therapy is promising with 7 out of 11 patients attaining complete remission, and CAR-NK cells were present in the body for up to a year. However, the most important outcome was in the safety results, the HLA-mismatched CAR-NK cells did not result in major toxic events such as GvHD, showing a future for the use of different cells in CAR therapy.

4 T cell activation and genetic modification

4.1 Background

After isolation from the patient, the T cells are activated by anti-CD3 and anti-CD28 antibodies which have a similar activating effect as antigen-presenting cells (Levine et al., 2017). These antibodies can be

supplemented in variable forms, such as soluble, on coated beads or with artificial antigen presenting cells, and can later be removed by magnetic separation (Larson & Maus, 2021). T cell activation optimizes the transduction efficiency of the viral vector encoding the CAR gene. Lentiviral and retroviral vectors are currently the most used vectors for genetically modifying T cells, with the ability to integrate CAR DNA permanently into the genome of the T cells (Levine et al., 2017). These viral vectors initiate long-term CAR expression and have a high transduction efficiency, quickly reaching the necessary amount of CAR-T cells (Zhang et al., 2017; De Godoy et al., 2022).

4.2 Bottlenecks

All currently approved CAR-T cell therapies use a viral vector to transduce the CAR gene in the T cells, which has been efficacious. However, the use of viral vectors is burdened by strict regulations and a demanding manufacturing process. The foremost reason for this is that retro- and lentiviral vectors have an oncogenic potential as they have a preference for DNA integration in active transcription sites of the host cell. Strict regulations are in place to ensure the safety of viral vectors, such as the obligation to assess the presence of replication-competent retrovirus (RCR) (Abdo et al., 2020). According to the FDA, patients should followed for 15 years to check for any RCRs causing adverse events. Aside from the strict regulations complicating production, the manufacturing of viral vectors is complex and can take several weeks, with only limited facilities manufacturing retroviral and lentiviral vectors (Levine et al., 2017). Furthermore, viral vectors are limited in size (~10 kb), which could be troublesome with new innovative CAR constructs (Lukjanov et al., 2021). Finally, for optimal transduction efficiency, T cells need to be activated before viral vector transduction, this adds two days to CAR-T cell production, while patients would benefit from a quicker production process (Abdo et al., 2020).

4.2.1 Expert opinion

Interviewed experts in the field of immunotherapy agree that the use of viral vectors is a bottleneck in the development of CAR-T cell therapy. An example is the difficulty of acquiring viral vector stock, with only limited vendors, the average waiting time is 1,5 years. Additionally, the strict regulations surrounding viral vectors further delay new CAR-T cell therapies to be used in the clinic. These bottlenecks hinder researchers in quickly developing new types of CAR-T cell therapies. This is why the future of CAR-T cell therapy lies in non-viral transfection of T cells, according to experts in the field.

4.3 Possible solutions

The future of CAR-T cell therapy could be transposons and mRNA technologies as possible non-viral transfection methods. These methods have a random or highly specific integration profile instead of the preference for transcriptionally active sites of viral vectors (Moretti et al., 2022). Non-viral vectors have a more straightforward manufacturing process which is not as strictly regulated compared to viral vectors. Additionally, most non-viral vectors are larger in size with the capability to transfect large or multiple CAR constructs for innovative CAR-T cell therapies (Irving et al., 2021).

Transposons have all the useful traits of viral vectors like prolonged gene expression without the limitations. Transposons are easier and cheaper to manufacture under GMP, have no size limitations, and have lower immunogenicity risks, making them a suitable target for CAR-T cell therapy optimization (Irving et al., 2021). Magnani et al. (2020) successfully created allogenic CAR-T cells which were genetically modified with a sleeping beauty transposon. Clinical trials showed positive antileukemic effects with almost all patients in complete remission without severe toxicities such as GvHD. In the majority of the patients, CAR-T cells were measurable for up to ten months, proving strong expansion *in vivo*. To show the safety of the sleeping beauty transposon, the integration site was analysed and confirmed a high safety profile. This study shows the possibilities of not only the sleeping beauty transposon but of allogenic CAR-T cell therapy as well.

Further optimization of CAR-T cell therapy is possible through specific gene integration, eliminating the risk of the oncogenic effect of random integration. CRISPR-Cas9 gene editing technology to produce CAR-T cells with specific gene integration is currently being used in multiple clinical trials. Lü et al. (2020) performed a clinical I trial to study the safety and feasibility of CRISPR edited CAR-T cells. The study showed that there were no off-target gene editing events in the patients and no severe adverse events, showing the safety of the therapy. However, in certain cases, this manufacturing process produced too few T cells, excluding e few patients from being treated. It is unclear if this was due to unfit T cells or the production process. It was also concluded that this gene editing therapy was less efficient than viral vectors. So while specific gene editing could be beneficial, more effective gene editing techniques are needed.

5 CAR-T cell expansion

5.1 Background

Following genetic modification, the CAR-T cells are cultured and stimulated with cytokines, mainly IL-2, to enhance proliferation (Sudarsanam et al., 2022). Current CAR-T cell therapies expand for 9-14 days, until they have reached the necessary therapeutic cell quantity (López-Cantillo et al., 2022).

5.2 Bottlenecks

As mentioned before in the bottlenecks of autologous T cell isolation, a long therapy production process is a disadvantage for patients with strongly progressive diseases. The duration of the CAR-T cell expansion is therefore the main bottleneck. Taking the whole production process into account, it is clear that the lengthy CAR-T cell therapy manufacturing process is due to the 9-14 days expansion.

Aside from the effect on the production process, long expansion has a negative effect on the quality of the T cells as well. Rapid and extensive proliferation of the T cells could lead to predominantly differentiated cells, which have a lesser anti-leukemic effect compared to naïve T cells. Naïve T cells are more likely to persist and proliferate *in vivo,* which is directly associated with a positive clinical response to CAR-T cell therapy (López-Cantillo et al., 2022).

Tackling this bottleneck is not only beneficial for optimal CAR-T cell therapy manufacturing, but also for a more efficient anti-tumour effect.

5.2.1 Expert opinion

Experts in the field of CAR-T cell therapy stated that while current therapies are effective, the CAR-T cell expansion is unnecessarily long and damages the T cell, diminishing the effect of the therapy. T-Charge™ of Novartis is an example of optimizing the expansion period. The CAR-T cells are expanded for only 3-5 days, allowing the cells to further proliferate *in vivo.* This resulted in a time efficient and cost efficient product, which showed superior anti-tumour effects compared to standard CAR-T cell therapies. The interviewed experts are convinced that rapid production of CAR-T cells can be achieved by limiting the expansion time, or even to completely expand *in vivo.*

5.3 Possible solutions

Ghassemi et al. (2018) studied the difference in differentiation and therapeutic effect of CAR-T cells harvested on day 3 compared to day 9. The study showed that the cells harvested at earlier time points were less differentiated and had improved antileukemic activity in mice models. The same research group tried to shorten the expansion time further based on the positive results, hypothesizing that not only expansion but activation as well was negatively influencing the effectiveness of the CAR-T cells (Ghassemi et al., 2022). The study aimed to create effective CAR-T cells within one day by eliminating the activation and expansion steps. The main limitation in achieving this goal was the low transduction efficiency in quiescent T cells. Therefore, transduction efficiency was optimized by serum starving the

cells before transduction, adding deoxynucleosides, and adjusting the surface area of the cell. The rapidly produced CAR-T cells were then compared to CAR-T cells which were produced the traditional way, showing increased potency in the non-activated and non-expanded CAR-T cells. To summarize, this study was able to create highly potent CAR-T cells within 24 hours, while simplifying and reducing the cost of the manufacturing process.

6 GMP and regulations

6.1 Background

The production of CAR-T cells must adhere to strict GMP regulations, ensuring that the final product is safe to administer to the patient. These regulations are applicable to every step of the CAR-T cell therapy manufacturing process, meaning the products being used, the personnel, the facility, and the equipment all need to comply with GMP. Furthermore, strict protocols, recordkeeping, and stability testing are needed for GMP compliance. (Gee, 2018).

6.2 Bottlenecks

Strict GMP regulations are in place to maintain the safety of the final product, however, they limit the efficiency of the CAR-T cell therapy manufacturing process in many ways. As previously mentioned, the use of viral vectors is strictly GMP regulated, restraining the use of these vectors (Lukjanov et al., 2021). Additionally, institutions willing to produce CAR-T cell therapy in-house are often hindered by GMP regulations resulting in only a few manufacturing centres being able to produce CAR-T cell therapy under GMP, limiting the availability (Gee, 2018). Only few published articles go into depth about the bottlenecks of GMP regulations on CAR-T cell production, therefore the experts working under GMP will highlight the bottlenecks of GMP and possible solutions.

6.2.1 Expert opinion

The interviewed experts on GMP are currently working at the Universitair Medisch Centrum Utrecht (UMCU). The UMCU is using a diverse team to bring CAR-T cell therapy from research to the clinic under GMP. The first bottleneck in this process is creating a protocol based on the research that has been done. Researchers usually do not specify which ranges are acceptable or note what happens if you deviate from protocol. Consequently, the GMP team needs to run/repeat many experiments to create a complete protocol. A bottleneck more specific to CAR-T cell therapy is the production time. To adhere to GMP standards, the protocol needs to be run perfectly three times in a row before production can start. The protocol for CAR-T cells takes 12 days, subsequently, three perfect runs would take over a month to complete. The experts stressed that this greatly hinders the efficiency, because with every failed run, they have to start over, extending the time it takes to bring the therapy to the patients.
Another expert mentioned how the strict regulations, besides GMP, hinder stimulating innovative ideas, for every small adjustment to the regulatory process starts over, which complicates the optimisation of existing therapies.

6.3 Possible solutions

A general solution to simplify translating research into the clinic is to bring awareness to the researchers to work more precisely and most importantly, to document every result, whether it is positive or negative. According to the experts, this could save a lot of time and resources in creating a protocol.
The extensive period in which the protocol needs to run three times perfectly could be greatly shortened by reducing the production time of CAR-T cell therapy, while still adhering to GMP. Additionally, the experts mentioned that adjustments in personnel and facilities could improve the lengthy protocol testing period. This project at UMCU is new, with mainly junior and inexperienced researchers executing the project, consequently, more seniors with expertise in GMP would be beneficial. Moreover, the

facilities are now limited to one run at a time, while the ability to run the protocol in parallel would considerably improve the efficiency of protocol testing. An important note from the experts is that while optimizing CAR-T cell therapy is beneficial to the patients and the manufacturers, it will need a new validation to comply with GMP, which takes years to complete.
Finally, the experts are advocating for flexibility in regulations regarding slight changes in therapies. Stewart et al. (2020) described that to stimulate innovation and accelerate development, it would be beneficial to adjust the strictness of regulations after making a risk mitigation assessment, while ensuring the safety of the patients.

7 New strategies: *In vivo* CAR-T cell production

Previously mentioned solutions were all focused on one step in the CAR-T cell therapy manufacturing process. These solutions are promising strategies, however, *ex vivo* manufacturing is prone to be a complex production process. That is why it is important to highlight new strategies that change CAR-T cell therapy completely.
Agarwalla et al. (2022) designed an implantable scaffold that is loaded with autologous T cells and retroviral particles which is implanted subcutaneously for *in vivo* release of CAR-T cells. The study showed that the scaffold released fully functional CAR-T cells with an increased anti-tumour efficacy compared to standard CAR-T cell therapy in mice. This technique bypasses the complicated *ex* vivo production process and reduces production time to a single day, tackling multiple bottlenecks in CAR-T cell therapy manufacturing.
Another technique gaining traction is nanocarriers containing CAR genes with a specificity for T cells. The T cells are induced *in vivo* by de nanocarriers, generating functional and effective CAR-T cells (Xin et al., 2022). For instance, Agarwal et al. (2019) used a lentiviral vector with a human CD8α chain to target T cells *in vivo*. This resulted in potent CAR-T cells in mice which were able to eliminate the tumour cells completely. Furthermore, the lentiviral vector also transduced natural killer T cells and NK cells, potentially aiding the anti-tumour effect of CAR-T cells. These results are promising, however, the use of viral vectors is not optimal as discussed before. Parayath et al. (2020) designed biodegradable polymeric nanocarriers carrying CAR mRNA. The nanocarriers were enhanced with anti-CD8 antibodies to target T cells primarily. The study showed that the nanocarriers were able to transfect T cells efficiently with CAR mRNA, resulting in a similar anti-tumour effect as standard CAR-T cell therapy. An advantage of using mRNA nanocarriers is that the design is easily modifiable and the manufacturing process is relatively easy and quick.
These nanocarriers simplify the manufacturing process considerably, they are easily standardized, there is no need for isolation of autologous material, no *ex vivo* expansion, and development is less limited by GMP.
Finally, Rurik et al. (2022) showed that *in vivo* CAR-T cell therapy could be applicable beyond the scope of haematological malignancies. The study designed mRNA lipid nanoparticles that target T cells to produce anti-fibrotic CAR-T cells *in vivo* to treat cardiac injury. These nanoparticles were tested on a mouse model with heart failure, resulting in effective CAR-T cells which reduced fibrosis and restored cardiac activity. This study shows the potential of CAR-T cell therapy, not only for cancer, but for other types of diseases as well.

8 Discussion

CAR-T cell therapy has emerged as a promising cancer immunotherapy that harnesses a patient's own immune system to effectively treat haematological malignancies. However, the production process of CAR-T cell therapy is time-consuming and complicated. This review explored the bottlenecks in the production process, based on literature and interviews with experts in the field of CAR-T cell therapy.

Every step of the production process has its specific bottlenecks, however, the most limiting bottlenecks can be categorised under two overarching bottlenecks: Time and Regulation. CAR-T cells take weeks to produce, while patients who would benefit from CAR-T cell therapy are critically ill and often have highly progressive malignancies. The bottleneck in the use of autologous material and the expansion of T cells is essentially time. The use of autologous material limits the ability to have an off-the-shelf therapy since the production process only starts when autologous T cells are isolated from the patient. The expansion of T cells adds an extra 9-14 days to the production process. Therefore, production time is a main bottleneck since patients simply do not have the time to wait weeks for an effective treatment. The regulations associated with CAR-T cell therapy is the other main bottleneck identified. The use of viral vectors is standard in transducing T cells, however, manufacturers are limited by strict regulations due to safety concerns. GMP regulations are another type of regulation limiting manufacturers due to the complexity and high cost of the process. Consequently, only a few manufacturing centres are able to comply with the regulations, greatly limiting the availability of CAR-T cell therapy for patients.

Possible solutions and new strategies were explored based on the identified bottlenecks and are depicted in Table 1. Allogeneic material for CAR-T cell therapy could potentially eliminate the waiting time for patients, creating an off-the-shelf therapy. Currently, research is being done to overcome the safety concerns by genetically modifying the allogenic T cells or using NK cells instead of T cells. The expansion time and the use of viral vectors could possibly be improved in a similar way as it can be improved for autologous CAR-T cell therapy. An important bottleneck not overcome by allogeneic material is the GMP regulation since those will be similar to autologous material. Nevertheless, this is a promising strategy that is also beneficial when autologous material is too damaged or depleted. The use of transposons is a smart way to circumvent the use of viral vectors and the associated strict regulations, and improve production time by limiting the wait time for the production of viral vectors. However, the effect of this solution is limited to the bottlenecks of viral vectors, but could possibly be used in combination with other solutions, such as allogenic CAR-T cell therapy for better optimization of CAR-T cell therapy. By skipping T cell activation and reducing expansion time, CAR-T cells can be produced within 24 hours, greatly shortening production time. This solution is mostly beneficial when using autologous material since the time between isolation and re-infusion is critical. A completely different strategy would be *in vivo* CAR-T cell production, either using scaffolds or nanocarriers. This strategy eliminates the need for ex vivo manufacturing altogether, thereby reducing the time and complexity of the production process since scaffolds and nanocarriers are relatively easily produced under GMP. A notable difference between scaffolds and nanocarriers is that scaffolds use viral vectors to transduce T cells. Consequently, *in vivo* CAR-T cell production by nanocarriers is the only strategy able to overcome the four main bottlenecks.

Table 1: Overview of the effect of the solutions on identified bottlenecks, compared to standard CAR-T cell therapy.

	Time		Regulation	
Solutions	Expansion time	Production time	Viral vectors	GMP
Allogeneic material	~	++	~	=
Transposon	=	~	++	=
Shortening expansion	++	++	=	=
Scaffold *in vivo*	++	++	=	++
Nanocarriers *in vivo*	++	++	++	++

++ Greatly improves
+ Improves
~ Potentially improves
= No difference

Notably, the availability of Advanced Therapy Medicinal Products (ATMPs), which includes CAR-T cell therapy, is under discussion. New EU pharmaceutical legislation expands the hospital exemption legislation, which previously was used to allow hospitals to produce a treatment in exceptional situations for an individual patient. However, the new legislation gives hospitals the ability to produce ATMPs. Hospital pharmacists and research institutes are positive about this development, viewing it as an opportunity to bridge the gap between academia and the clinic (*Hospital Exemption in ATMP development*, 2023). However, organisations siding with the pharmaceutical industry are opposed to the new legislation, worrying that academia cannot unlock the potential of ATMPs alone (*Joint Position Paper on the Hospital Exemption Scheme for ATMPs*). Currently, the European Medicines Agency is conducting a pilot to establish the effectiveness of this new legislation and what needs need to be met still. Nevertheless, it is important to state that the logistical, financial, and regulatory benefits of allowing hospitals to produce ATMPs, greatly improve the availability of these treatments and thus the availability of CAR-T cell therapy.

This review shows that CAR-T cell therapy has many bottlenecks and can therefore be optimized in many ways. However, *in vivo* CAR-T cell production with nanocarriers has shown the greatest potential in overcoming the identified bottlenecks. This strategy reduces production time and costs, has fewer regulations and could be readily available for patients. An important aspect to note is that a patient needs enough healthy T cells to benefit from this therapy which is why it is important to keep optimizing other strategies. Consequently, the combination of allogeneic CAR-T/NK cell therapy with transposons instead of viral vectors could be a promising solution since it bypasses most bottlenecks and is not dependent on the quality or the number of autologous T cells. Finally, improving availability of CAR-T cell therapy by allowing hospitals to produce the therapy in-house could be the extra step needed so that these strategies can revolutionize the field of CAR-T cell therapy.

9 References

Abdo, L., Barros, L. R. C., Viegas, M. S., Marques, L. V. C., De Sousa Ferreira, P., Chicaybam, L., & Bonamino, M. H. (2020). Development of CAR-T cell therapy for B-ALL using a point-of-care approach. OncoImmunology, 9(1). https://doi.org/10.1080/2162402x.2020.1752592

Agarwal, S., Weidner, T., Thalheimer, F. B., & Buchholz, C. J. (2019). In vivo generated human CAR T cells eradicate tumor cells. OncoImmunology, 8(12), e1671761. https://doi.org/10.1080/2162402x.2019.1671761

Agarwalla, P., Ogunnaike, E. A., Ahn, S., Froehlich, K., Jansson, A., Ligler, F. S., Dotti, G., & Brudno, Y. (2022). Bioinstructive implantable scaffolds for rapid in vivo manufacture and release of CAR-T cells. Nature Biotechnology, 40(8), 1250–1258. https://doi.org/10.1038/s41587-022-01245-x

Blache, U., Popp, G., Dünkel, A., Koehl, U., & Stephan, F. (2022). Potential solutions for manufacture of CAR T cells in cancer immunotherapy. Nature Communications, 13(1). https://doi.org/10.1038/s41467-022-32866-0

Caruso, H. G., Tanaka, R., Liang, J., Ling, X., Sabbagh, A., Henry, V., Collier, T. L., & Heimberger, A. B. (2019). Shortened ex vivo manufacturing time of EGFRVIII-specific chimeric antigen receptor (CAR) T cells reduces immune exhaustion and enhances antiglioma therapeutic function. Journal of Neuro-Oncology, 145(3), 429–439. https://doi.org/10.1007/s11060-019-03311-y

Chen, L., Xie, T., Wei, B., & Di, D. (2022). Current progress in CAR-T cell therapy for tumor treatment (Review). Oncology Letters, 24(4). https://doi.org/10.3892/ol.2022.13478

De Godoy, J. A. P., Bonamino, M., Paiva, R. M. A., Kondo, A. T., Okamoto, O. K., & Kerbauy, L. N. (2022). CAR-T cell production. Journal of Bone Marrow Transplantation and Cellular Therapy, 3(1), 155. https://doi.org/10.46765/2675-374x.2022v3n1p155

Depil, S., Duchâteau, P., Grupp, S., Mufti, G. J., & Poirot, L. (2020). 'Off-the-shelf' allogeneic CAR T cells: development and challenges. Nature Reviews Drug Discovery, 19(3), 185–199. https://doi.org/10.1038/s41573-019-0051-2

EPTRIAdmin. (2023, 17 augustus). Hospital Exemption in ATMP development- EPTRI. EPTRI. https://eptri.eu/news/hospital-exemption-in-atmp-development/

Eyquem, J., Mansilla-Soto, J., Giavridis, T., Van Der Stegen, S. J. C., Hamieh, M., Cunanan, K., Odak, A., Gönen, M., & Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/CAS9 enhances tumour rejection. Nature, 543(7643), 113–117. https://doi.org/10.1038/nature21405

Gee, A. P. (2018). GMP CAR-T cell Production. Best Practice & Research Clinical Haematology, 31(2), 126–134. https://doi.org/10.1016/j.beha.2018.01.002

Ghassemi, S., Durgin, J. S., Nuñez-Cruz, S., Patel, J. R., Leferovich, J., Pinzone, M. R., Shen, F., Cummins, K. D., Plesa, G., Cantu, A., Reddy, S., Bushman, F. D., Gill, S., O'Doherty, U., O'Connor, R. S., & Milone, M. C. (2022). Rapid manufacturing of non-activated potent CAR T cells. Nature Biomedical Engineering, 6(2), 118–128. https://doi.org/10.1038/s41551-021-00842-6

Ghassemi, S., Nuñez-Cruz, S., O'Connor, R. S., Fraietta, J. A., Patel, P., Scholler, J., Barrett, D. M., Lundh, S., Davis, M. M., Bedoya, F., Zhang, C., Leferovich, J., Lacey, S. F., Levine, B. L., Grupp, S. A., June, C. H., Melenhorst, J. J., & Milone, M. C. (2018). Reducing Ex vivo culture improves the antileukemic activity of chimeric antigen receptor (CAR) T cells. Cancer immunology research, 6(9), 1100–1109. https://doi.org/10.1158/2326-6066.cir-17-0405

Irving, M., Lanitis, E., Migliorini, D., Ivics, Z., & Guedan, S. (2021). Choosing the right tool for genetic engineering: Clinical lessons from Chimeric Antigen Receptor-T Cells. Human Gene Therapy, 32(19–20), 1044–1058. https://doi.org/10.1089/hum.2021.173

Joint Position Paper on the Hospital Exemption Scheme for ATMPs. (z.d.). https://www.efpia.eu/news-events/the-efpia-view/efpia-news/joint-position-paper-on-the-hospital-exemption-scheme-for-atmps/

Larson, R. C., & Maus, M. V. (2021). Recent advances and discoveries in the mechanisms and functions of CAR T cells. Nature Reviews Cancer, 21(3), 145–161. https://doi.org/10.1038/s41568-020-00323-z

Levine, B. L., Miskin, J., Wonnacott, K., & Keir, C. H. (2017). Global manufacturing of CAR T cell Therapy. Molecular Therapy- Methods & Clinical Development, 4, 92–101. https://doi.org/10.1016/j.omtm.2016.12.006

Lin, H., Cheng, J., Mu, W., Zhou, J., & Zhu, L. (2021). Advances in universal CAR-T cell therapy. Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.744823

Liu, E., Marín, D., Banerjee, P. P., Macapinlac, H. A., Thompson, P. A., Basar, R., Kerbauy, L. N., Overman, B. J., Thall, P. F., Kaplan, M., Nandivada, V., Kaur, I., Cortes, A. N., Cao, K., Daher, M., Hosing, C., Cohen, E. N., Kebriaei, P., Mehta, R. S., . . . Rezvani, K. (2020). Use of CAR-Transduced natural killer cells in CD19-Positive lymphoid tumors. The New England Journal of Medicine, 382(6), 545–553. https://doi.org/10.1056/nejmoa1910607

López-Cantillo, G., Urueña, C., Camacho, B., & Ramírez-Segura, C. (2022). CAR-T cell performance: How to improve their persistence? Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.878209

Lü, Y., Xue, J., Deng, T., Zhou, X., Yu, K., Deng, L., Huang, M., Yi, X., Liang, M., Wang, Y., Shen, H., Tong, R., Wang, W., Li, L., Song, J. S., Li, J., Su, X., Ding, Z., Gong, Y., . . . Mok, T. (2020). Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nature Medicine, 26(5), 732–740. https://doi.org/10.1038/s41591-020-0840-5

Lukjanov, V., Koutná, I., & Šimara, P. (2021). CAR T-Cell production using nonviral approaches. Journal of immunology research, 2021, 1–9. https://doi.org/10.1155/2021/6644685

Lv, Z., Luo, F., & Chu, Y. (2023). Strategies for overcoming bottlenecks in allogeneic CAR-T cell therapy. Frontiers in Immunology, 14. https://doi.org/10.3389/fimmu.2023.1199145

Magnani, C. F., Gaipa, G., Lussana, F., Belotti, D., Gritti, G., Napolitano, S., Matera, G., Cabiati, B., Buracchi, C., Borleri, G., Fazio, G., Zaninelli, S., Tettamanti, S., Cesana, S., Colombo, V., Quaroni, M., Cazzaniga, G., Rovelli, A., Biagi, E., . . . Biondi, A. (2020). Sleeping beauty–engineered CAR T cells achieve antileukemic activity without severe toxicities. Journal of Clinical Investigation, 130(11), 6021–6033. https://doi.org/10.1172/jci138473

Moretti, A., Ponzo, M., Nicolette, C. A., Tcherepanova, I. Y., Biondi, A., & Magnani, C. F. (2022). The past, present, and future of Non-Viral CAR T cells. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.867013

Nawaz, W., Huang, B., Xu, S., Li, Y., Zhu, L., Hu, Y., Wu, Z., & Wu, X. (2021). AAV-mediated in vivo CAR gene therapy for targeting human T-cell leukemia. Blood Cancer Journal, 11(6). https://doi.org/10.1038/s41408-021-00508-1

Parayath, N. N., Stephan, S. B., Koehne, A., Nelson, P. S., & Stephan, M. T. (2020). In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in vivo. Nature Communications, 11(1). https://doi.org/10.1038/s41467-020-19486-2

Rurik, J. G., Tombácz, I., Yadegari, A., Fernández, P. O. M., Shewale, S. V., Li, L., Kimura, T., Soliman, O. Y., Papp, T. E., Tam, Y. K., Mui, B. L., Albelda, S. M., Puré, E., June, C. H., Aghajanian, H., Weissman, D., Parhiz, H., & Epstein, J. A. (2022). CAR T cells produced in vivo to treat cardiac injury. Science, 375(6576), 91–96. https://doi.org/10.1126/science.abm0594

Sterner, R. C., & Sterner, R. M. (2021). CAR-T cell therapy: current limitations and potential strategies. Blood Cancer Journal, 11(4). https://doi.org/10.1038/s41408-021-00459-7

Stewart, M., Keane, A. T., Butterfield, L. H., Levine, B. L., Thompson, B., Xu, Y., Ramsborg, C., Lee, A., Kalos, M., Koerner, C., Moore, T. D., Markovic, I., Lasiter, L., Ibrahim, R., Bluestone, J. A., Sigal, E. V., & Allen, J. (2020). Accelerating the development of innovative cellular therapy products for the treatment of cancer. Cytotherapy, 22(5), 239–246. https://doi.org/10.1016/j.jcyt.2020.01.014

Sudarsanam, H., Buhmann, R., & Henschler, R. (2022). Influence of culture conditions on ex vivo expansion of T lymphocytes and their function for therapy: current insights and open questions. Frontiers in Bioengineering and Biotechnology, 10. https://doi.org/10.3389/fbioe.2022.886637

Xin, T., Cheng, L., Zhou, C., Zhao, Y., Hu, Z., & Wu, X. (2022). In-Vivo induced CAR-T cell for the potential breakthrough to overcome the barriers of current CAR-T cell therapy. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.809754

Zhang, C., Li, J., Zhong, J., & Zhang, X. (2017). Engineering CAR-T cells. Biomarker research, 5(1). https://doi.org/10.1186/s40364-017-0102-y

10 Consulted experts

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