CAR-T cell therapy for cancer

Applications of Genetic Engineering and Biotechnology.

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Class 3b
17.5.2024

Contents

Preface

In recent years, the field of cancer therapy has witnessed a remarkable evolution, underscored by the integration of cutting-edge biomedical technologies that promise to redefine traditional treatment paradigms. Among these, Chimeric Antigen Receptor (CAR) T-cell therapy stands out as a revolutionary approach, offering new hope to patients with previously intractable forms of cancer. Our motivation for delving into this topic stems from a deep fascination of immunology and oncology, where science meets a profound human need. Besides that, we both lost family members to cancer, so this topic awakes great interest in us. This term paper aims to unpack the complex science behind CAR-T cell therapy, illuminate its transformative potential, and address the challenges it faces on the path to broader clinical application.

What makes CAR-T cell therapy especially interesting is its unique mechanism of action. Unlike conventional therapies that often employ external agents such as chemicals or radiation, CAR-T cell therapy harnesses the body's own immune system to fight cancer. By genetically modifying T-cells to express specific receptors, scientists can empower the immune system to recognize and destroy cancer cells with precision. This personalized approach not only targets cancer cells more effectively but also opens the door to treatments tailored to the individual genetic makeup of each patient's cancer.

Moreover, the success stories emerging from early clinical trials have been nothing short of extraordinary, particularly in treating certain types of leukemia and lymphoma where other therapies have failed. These outcomes not only underscore the potential of CAR-T cell therapy as a powerful weapon against cancer but also highlight the scientific and medical insight driving its development. [1]

Introduction

CAR-T cell therapy, a transformative approach in the battle against cancer, harnesses the body's own immune defenses by genetically modifying T-cells to target and eliminate cancer cells. CARs are artificially produced receptors that act to direct lymphocytes. Their circumvention of the requirement for major histocompatibility complex (MHC) dependent antigen presentation and subsequent activation makes them ideal cancer targeting agents as many cancer cells actively downregulate MHC I expression to avoid immune surveillance. This innovative treatment has garnered significant attention due to its potential to treat cancers that are resistant to conventional therapies. In this context, the therapy is particularly utilized in cases of lymphomas and certain leukemias, where traditional treatment options have been exhausted. The urgency and necessity of this intervention lie in its ability to provide a potentially lifesaving option for patients with few or no alternative treatments available.

From 1989, when scientists first introduced the concept of ‘CAR’ in human history, to 2022, when the FDA approved B-cell maturation antigen CAR-T cells for the treatment of multiple myeloma, it has been more than 30 years since the development of CAR-T immune cell therapy. CAR-T immune cell therapy has helped some patients with haematologic cancers achieve remissions of up to 10 years and future breakthroughs in the field of CAR-T cells are sure to follow, with major trends in the field involving targets, disease treatment modalities and more.

The primary reason for employing CAR T-cell therapy lies in its targeted approach. By modifying the patient's T-cells to recognize and attack tumor cells specifically, this therapy can achieve results unattainable with more traditional methods. It is currently approved for use in specific types of blood cancers, such as lymphomas and a subgroup of leukemias, that express the CD19 antigen. This specificity underlines the therapy's effectiveness, as these genetically engineered T-cells are adept at reducing tumor cells in these conditions.

Despite its promising results, CAR-T cell therapy is not without alternatives or challenges. The high costs associated with the therapy, driven by the complexities of cell modification and expansion, often limit its accessibility. The cells must be sent to specialized facilities for genetic modification and proliferation, a process that not only incurs significant expense but also logistical challenges. Furthermore, alternatives such as virus-free systems and CRISPR technology are being explored to mitigate risks and reduce costs. These newer methods promise to streamline the process by eliminating the need for viral vectors, which carry their own risks and complexities.

The landscape of cancer treatment is continually evolving, with CAR-T cell therapy at the forefront of these advancements. [1] [3] [6]

Description of the engineering technique

CAR-T cell therapy involves a sophisticated engineering process that modifies a patient's T-cells to specifically target and destroy cancer cells. This technique, used primarily in the treatment of certain blood cancers like lymphomas and specific leukemias, hinges on a series of intricate steps designed to reprogram the immune cells at a genetic level.

1. **Cell Extraction and Selection**: The process begins with the extraction of T-cells from the patient's blood. These cells are then selected and isolated to ensure that the most effective cells are used for engineering.
2. **Genetic Modification**: Once isolated, the T-cells are genetically modified using a viral vector, typically a lentivirus or retrovirus. This vector introduces a new gene that encodes for a specific chimeric antigen receptor (CAR) that can recognize a tumor antigen. This receptor is different from the normal T-cell receptor as it is designed to specifically bind to antigens present on the surface of the cancer cells.
3. **Activation and Expansion**: After genetic modification, the T-cells undergo activation and are cultured in bioreactors where they are expanded to produce millions of copies. This expansion is crucial as it ensures there are enough CAR-T cells to effectively target the cancer throughout the body.
4. **Quality Control**: Post-expansion, the cells undergo rigorous quality control checks to ensure they meet safety and efficacy standards. This step is vital to prevent potential adverse effects once the cells are reintroduced into the patient.
5. **Reinfusion into the Patient**: The engineered T-cells are then infused back into the patient, where they seek out and destroy cancer cells that express the specific antigen they were engineered to target. [3] [4] [6]

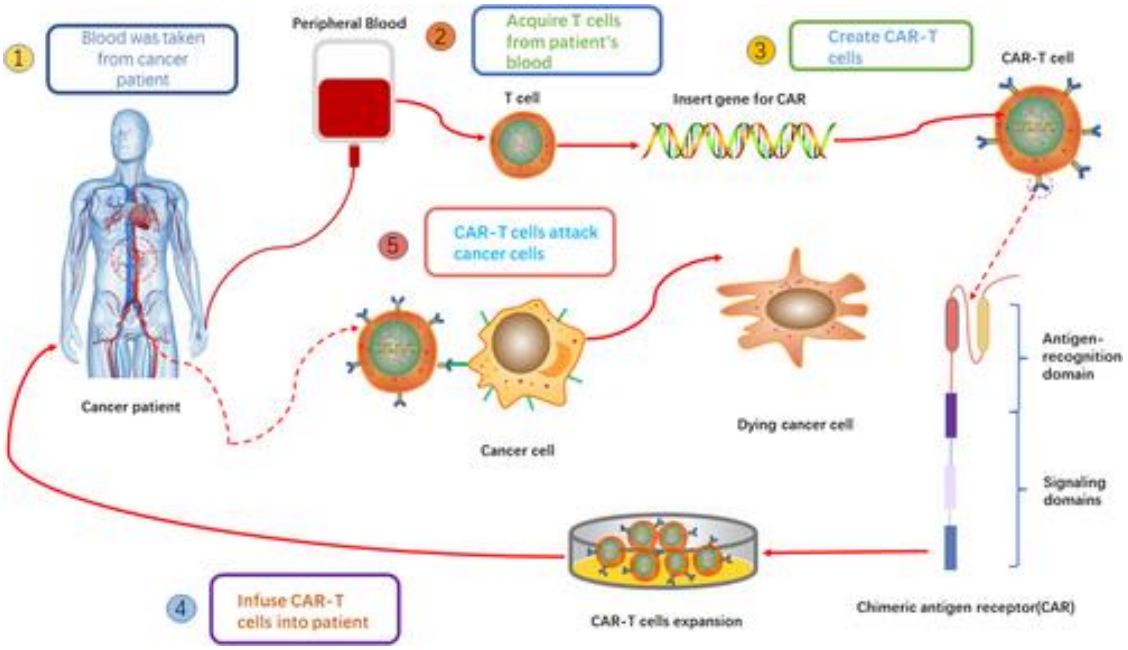

Figure 1: CAR-T cell therapy flow

CAR structure

CARs have a modular design with four major components: an antigen-binding domain, a hinge, a transmembrane domain and an intracellular signalling domain. Each of these elements has a distinct function and, optimal molecular design of the CAR can be achieved through many variations of the constituent protein domains.

Antigen recognition and binding domains

The antigen-binding domain of a CAR-T cell is the extracellular portion responsible for recognizing the target antigen and redirecting the specificity of CAR-expressing lymphocytes. Traditionally, these domains are composed of the variable heavy (VH) and variable light (VL) chains of monoclonal antibodies, connected by a flexible linker to form a single-chain variable fragment (scFv). The most common linker is the (Gly4Ser)3 peptide, which provides flexibility and solubility, resulting in a properly folded scFv capable of antigen recognition and binding. These scFvs typically target extracellular antigens on cancer cell surface proteins, enabling MHC-independent T-cell activation. Some CARs are designed with scFvs that can bind to soluble ligands in the tumor microenvironment, converting immunosuppressive signals into T-cell activators. Additionally, scFv sequences can be derived from murine or human monoclonal antibodies, or smaller single-domain antibodies (nanobodies) from camelid heavy-chain antibodies.

Hinge and transmembrane domains

The hinge and transmembrane domains of CARs connect the extracellular antigen-binding domain to the intracellular signalling domains. The hinge provides flexibility to overcome steric hindrance and sufficient length to access the target antigen, with variations in length and composition affecting antigen binding, signalling, cytokine production, and activation-induced cell death (AICD). Spacer sequences in the hinge domain help access membrane-proximal epitopes but may reduce CAR-T cell function. The transmembrane domain anchors the CAR in the T cell membrane and influences its stability and function.

Intercellular signalling domains

The intracellular signalling domain of a CAR typically includes an activation domain and one or more co-stimulatory domains. Most CARs activate CAR-T cells via immunoreceptor tyrosine-based activation motifs. However, these motifs alone are insufficient for a robust T cell response, necessitating additional co-stimulatory signals for optimal T cell function, metabolism, and persistence. CARs with co-stimulatory domains can proliferate upon repeated antigen exposure, leading to improved T cell persistence and activity. These domains are key to enhancing the efficacy and safety of CAR-T cell therapies and broadening their applicability to various cancers.

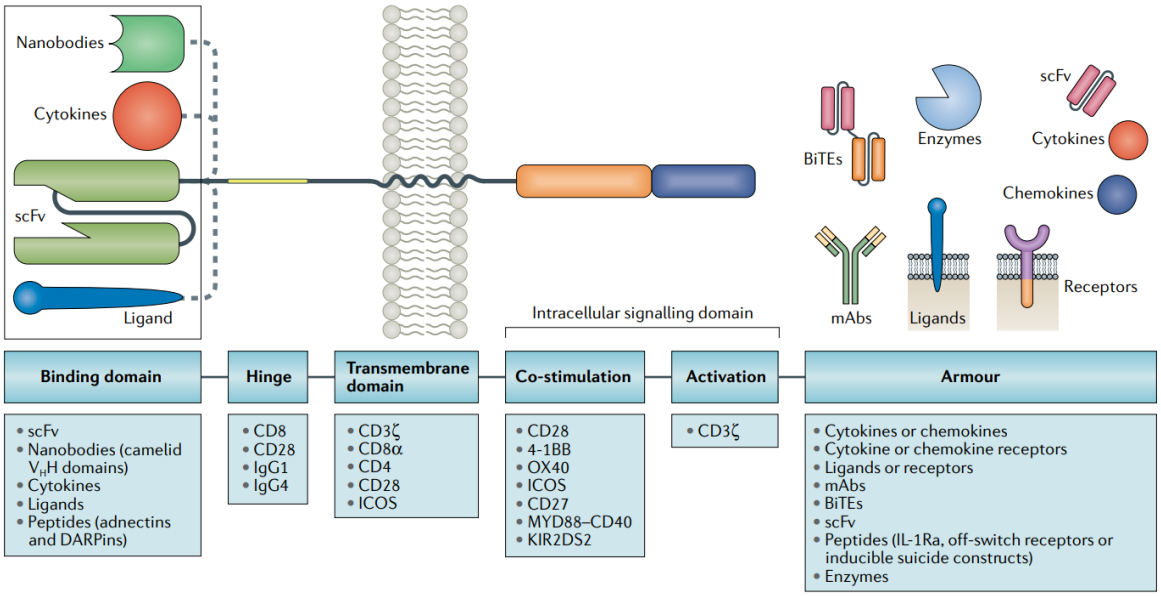

Figure 2: Blueprint of CAR design

[1] [3] [6]

Overcoming treatment related toxicities

Therapeutic responses to CAR-T cells in patients who otherwise have limited treatment options have been dramatic in some trials. These toxicities have been best characterized in patients treated with CD19-directed CAR-T cells, which were among the earliest CAR-T cell therapies used in successful clinical trials and the first to gain FDA approval. Mechanistically, the major CAR-T cell toxicities can be divided into two categories:
1) general toxicities related to T cell activation and subsequent systemic release of high levels of cytokines
2) toxicities resulting when CAR T cells target antigens that are also present on non-cancerous cells.

Cytokine release syndrome

To overcome the toxicity associated with systemic cytokine levels in CAR-T cell therapy, several strategies have been employed. Management of cytokine release syndrome (CRS) typically involves treatment with the anti-IL-6 receptor antibody tocilizumab, the anti-IL-6 antibody siltuximab, or corticosteroids.

On target-off target toxicity

To minimize on-target, off-tumor effects in CAR-T cell therapy, several strategies are employed. Optimizing antigen selection involves targeting antigens that are more specific to tumors or utilizing multiple antigens through logic-gated CARs, which ensures that CAR-T cells are activated only in the presence of multiple tumor-specific markers. Affinity tuning is another approach where CARs are designed with lower affinity to selectively target high-antigen-density tumor cells while sparing normal tissues, reducing the likelihood of damaging healthy cells. [2] [4] [5]

Interview

Figure 3: Prof. Dr. Gregor Hutter

We had the pleasure to interview Prof. Dr. Gregor Hutter, a senior neurosurgeon at University Hospital Basel specializing in neuro-oncology and is also a molecular immunologist. Dr. Hutter established his research group in Basel in 2018 focusing on developing combinatorial therapeutic approaches that locally target microglia and the adaptive immune system, and directly interfere with the tumor cells.
Through this in-depth interview we explored all aspects on the topic of CAR-T cell therapy and learned a lot. [6] [7]

Recent Advancements in CAR-T cell Therapy Research:
The researcher highlighted several recent advancements in CAR-T cell therapy. These include the development of "armored" CAR-T cells that are engineered to secrete cytokines or express ligands that enhance their effectiveness and persistence. Additionally, there has been significant progress in extending the application of CAR-T cell therapy to solid tumors, with new targeting strategies to overcome the tumor microenvironment's suppressive effects. [7]

Patient Evaluation for CAR T-Cell Therapy:
When asked how patients are evaluated for CAR-T cell therapy, the expert explained that candidates are typically those who have relapsed or refractory cancer after standard treatments, such as chemotherapy or radiation, have failed. Patients undergo comprehensive immunophenotyping to ensure their T-cells can be engineered and will respond to the modification. The presence of specific antigens, like CD19 in B-cell malignancies, is crucial for the therapy's targeting mechanism. [7]

Clinical Success Stories and Challenges:
From their own research and clinical practice, the researcher recounted success stories where patients achieved remission in cases where other treatments had failed. However, they also noted significant challenges such as cytokine release syndrome and difficulties targeting solid tumors due to the immunosuppressive tumor microenvironment. They are currently working on a novel CAR-T cell product targeting glioblastomas, specifically focusing on a tumor-specific antigen, EGFRV Three. This innovative approach not only targets tumor cells but also reprograms the tumor's immune environment by activating macrophages to attack remaining cancer cells. This "double strategy" has shown promising results in preclinical models and is moving towards human trials. [7]

Figure 4: Operation room at Uni hospital Basel

Challenges and Limitations of CAR T-Cell Therapy:

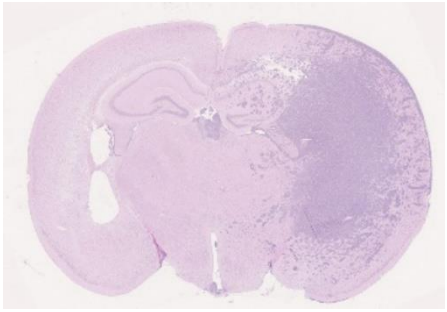

Figure 5: Glioblastoma

Despite its successes, CAR-T cell therapy faces several challenges. The researcher mentioned issues such as cytokine release syndrome (CRS), a potentially severe side effect, and the difficulty in targeting solid tumors due to the immunosuppressive nature of the tumor microenvironment, which simply means that the penetration of the tumor stroma provides the first obstacle to therapeutic efficacy and once CAR-T cells have penetrated into the tumor microenvironment, multiple additional levels of suppression are present that will impact the ability of the CAR-T cells to effect anti-cancer activity. A meta-analysis of 22 studies also showed that the response rate of CAR-T therapies in solid tumors was only 9%. The complexity and cost of manufacturing and delivering these personalized therapies also pose significant barriers. [7]

Future of CAR T-Cell Therapy:

Looking forward, the researcher was optimistic about the future of CAR-T cell therapy. Ongoing research focuses on enhancing the safety and efficacy of these treatments, with developments in gene editing technologies like CRISPR and TALEN offering new possibilities for refining CAR-T cell design. Another promising area is the development of off-the-shelf CAR-T cell products, which could significantly reduce costs and improve accessibility. [7]

Figure 6: Clinical lab at Uni hospital Basel

Accessibility of CAR T-Cell Therapies:

The high costs associated with CAR-T cell therapies remain a significant concern. The researcher advocated for global efforts to reduce costs through innovations in manufacturing and logistics, as well as policy changes to support broader insurance coverage and access to these potentially life-saving treatments. [7]

Discussion

CAR-T cell therapy has shown considerable progress in treating hematologic cancers like lymphoma and leukemia, including acute myeloid leukemia (AML). This innovative approach, which genetically modifies a patient's T-cells to target and destroy cancer cells, has demonstrated considerable success in cases where traditional therapies have failed. Notably, remission rates for these typically intractable cancers have been promising, leading to several CAR-T cell therapies receiving FDA approval. The expansion of this technique into trials for solid tumors and the development of new generations of CAR-T cells, such as "armored" CARs that secrete therapeutic agents or have built-in safety switches, highlight the rapid progress in this area. Recent advancements specifically in targeting AML have centered on identifying and validating new targets for immunotherapy, optimizing CAR designs, and reducing adverse effects. These efforts have paved the way for CAR-T cell therapies to begin addressing the unique challenges presented by AML, such as the heterogeneity of antigen expression among patients and the preservation of normal hematopoietic stem cells while targeting malignant ones.

What the Future holds for CAR-T cell therapy

While current results are encouraging, extensive research is needed to overcome existing challenges and enhance the efficacy and safety of CAR-T cell therapies. Future research will likely focus on:

1. **Improving Targeting Efficacy**: Developing CAR-T cells that can more effectively target solid tumors, overcoming the immunosuppressive microenvironment these tumors create.
2. **Reducing Costs and Enhancing Accessibility**: Innovations in manufacturing and delivery processes are crucial to reduce the therapy's cost and increase its availability to a broader patient population.
3. **Minimizing Adverse Effects**: Research into managing and preventing severe side effects, such as cytokine release syndrome and neurotoxicity, is essential.
   Exploring Combination Therapies: Combining CAR-T cell therapy with other treatments such as checkpoint inhibitors or traditional chemotherapy may enhance efficacy and overcome resistance. [2] [4] [5] [7]

Ethical Aspects of CAR-T cell therapy

Category	Aspect	Description
Strengths	Personalized Treatment	Utilizes genetically modified cells to target only cancerous cells, minimizing impact on healthy cells and improving efficacy.
	Durable Responses	Can lead to long-term remission in hematologic cancers, especially where other treatments have failed.
Weaknesses	High Cost	Prohibitively expensive, limiting access to a small subset of patients, potentially widening health disparities.
	Severe Side Effects	Can induce severe side effects such as cytokine release syndrome (CRS) and neurotoxicity, which can be lethal if not managed properly.
Threats	Equity of Access	The high cost and complex logistics could exacerbate health disparities.
	Ethical Concerns	Raises concerns about long-term effects and potential germline transmission if the therapy is broadened.
Opportunities	Patients with Refractory Cancer	Provides a new hope for remission and potentially a cure for patients who have not responded to conventional treatments.
	Advancements in Genetic Engineering	Pushes the boundaries of genetic engineering, opening doors for its application in other diseases.

CAR-T cell therapy, as outlined in the previous table, represents a relatively new and evolving treatment approach, with its unique strengths and notable challenges. Although the therapy currently faces issues such as high costs and severe side effects, ongoing advancements in medical technology and research offer considerable hope. As the field progresses, we can expect many of these downsides to be significantly reduced or even eliminated. This evolution will potentially enhance the therapy's effectiveness and expand its accessibility, making it a more viable option for a broader range of patients in the near future. [2] [4] [5] [7]

Summary

In this term paper, we've delved into the transformative world of CAR-T cell therapy, a cutting-edge approach in cancer treatment that leverages the body's own immune cells to fight cancer with extreme precision. By genetically engineering T-cells to attack cancer cells, this therapy has shown remarkable success, particularly in treating stubborn cases of leukemia and lymphoma that have resisted traditional treatments. Despite its potential, CAR-T cell therapy is not without its hurdles, including high costs, logistical challenges, and serious side effects like cytokine release syndrome. However, with ongoing advances in gene editing and manufacturing, the future looks bright. This therapy could dramatically alter the landscape of cancer treatment, promising a new era of personalized medicine that is both effective and tailored to individual patient needs. [1] [2] [3] [4] [5] [6] [7]

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Reference [6]:
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Reference [7]:
Interview Prof. Dr. Gregor Hutter (in appendix)

Picture References

Use of AI

Chat GPT was used for correcting spelling errors and improving the general syntax of the whole paper.

*All sources were last checked at 18:00, 19.05.2024

Interview with Prof. Dr. Gregor Hutter Transcript:

Question: What are CAR T cells?

Answer: These are special genetically modified cells, i.e., T cells from the immune system, which are taken from the patient and transduced with a receptor that recognizes a tumor antigen. This receptor activates them intracellularly so that when the receptor that binds the antigen is active, it specifically destroys the tumor, independently of the normal TCA receptor.

Question: How is a patient generally assessed to determine if they are a suitable candidate for CAR T-cell therapy?

Answer: Currently, there are only two approved indications: one for lymphomas and one for a subgroup of leukemias, not yet for other tumors. These are mostly patients who no longer have any other valid types of therapy, i.e., where there is no alternative. Attempts are made to produce cells with a specific CD19 antigen, which can then reduce these tumor cells. So far, this has only been done in a few cases because it is very expensive and, as I said, only standard for a few types of tumor.

Question: If I understood correctly, T cells are taken from the patient, then specialized to target the tumor they have, and then reinjected?

Answer: Exactly, they are taken from patients, transduced with a virus (lentivirus or retrovirus) that encodes this gene, then they are sorted and expanded with various factors in a bioreactor. Quality control is carried out before they are returned to the patient.

Question: From your perspective, what are the challenges or limitations with the use of CAR T-cell therapy?

Answer: The challenge is that it's a very expensive therapy and logistically complex. The therapies currently approved are manufactured by Novartis, I believe, and then the cells are taken from the patient and sent to America where they are expanded. This takes about three weeks, and then they are sent back again. This is logistically relatively complex and also very expensive because it is carried out in a so-called GMP (Good Manufacturing Practice) unit, tested sterile, and so on, making it very expensive. A single treatment costs about half a million, I think, for the patient. It is expensive, but it also works very well. In some cases, great success has been achieved with these patients.

Question: Given the high costs, what would be an alternative for this therapy? Or is there any alternative?
Answer: Yes, at the moment these lentiviruses are being used. Lentiviruses are just viruses; they also have their own risks, which means that they can ultimately integrate into the genome. This can be dangerous depending on the situation, and producing lentiviruses in a

GMB setting makes it very expensive because additional quality criteria also count. That's why nowadays, people are moving more towards virus-free systems, i.e., where you get rid of the viruses, and you have a cleaner system without viruses. It's also cheaper; it only costs a fifth to a tenth of the lentivirus. The fur is now moving more in this direction. Then there's this CRISPR technology which is also on the rise. This will probably make everything much cheaper in the future if you simply combine it with simpler technologies. That's gene editing; I think it will become mainstream at some point. It's not mainstream at the moment but it will certainly become much more affordable in the future because these therapies are also on the rise.

Question: So, do you think that as these therapies become more popular, the price will naturally decrease because it won't be so specialized anymore?

Answer: Yes, I think so because oncology is now also moving towards solid tumors with CAR T cells. We are also trying to produce this ourselves in the hospital, not profit-based but simply. Yes, you can produce it yourself in hospitals because the patients are in the hospital and we have the cells. And we actually have GMP facilities which would also make the whole thing cheaper.

Question: You also mentioned the future. Do you think there will be major and promising advancements, or do you think it will stay the same now?

Answer: No, there will be massive progress. Until now, we have simply made mono-specific CAR Ts. There are now more and more biospecifics that attack several targets simultaneously, then there are these so-called armored CAR T cells that also secrete factors that, for example, repolarize the immune environment. There are inducible CAR T cells that still work if you know the day-to-day work, i.e., that can be switched on and off, so to speak, with various genetic tricks. Yes, the field is a huge playground of different strategies, and one or the other will prevail. Then there are the suicide switches where you can switch off the CAR T cells because that is a very effective therapy. Yes, you can also overshoot, which is, of course, a problem for patients. So from that point of view, there will be a lot of development. There won't just be CAR T cells; there will also be CAR macrophages or modified macrophages, and so on—the stem cell area will be modified and so on—all of this will develop massively.

Question: What influence do government agencies like the FDA have on the development of such therapies, given the risks to patients?

Answer: Regulatory function is also becoming more complicated. It takes a lot of effort to even enter the clinic. It requires various preclinical studies, which are very time-consuming—first in animals, then perhaps even in non-human primates, although I don't think that's required anymore. However, the validation and safety issues are already quite significant, although it's now slowly becoming mainstream, as I said. And patients' own cells are being used, which means there is a certain degree of safety here because it matches the same patient. It's simply a transplant, an autologous blood donation, so to

speak. Of course, the cells are modified and, depending on the target, need certain safety measures that the regulatory authorities want to check and know about. And then you have to go through these phases of clinical trials, which are time-consuming and expensive, but that's actually the case with every drug in the end.

Question: What are the risks of this cell therapy, if there are any? Are there really severe risks for the patient?

Answer: Yes, there are risks. I mean, CD19, for example, is not only expressed on the tumor cells but also on others, for example, on the B cells, and the normal ones. They then, of course, also attack the normal B cells. In other words, you end up with no more B cells. These T cells are still there. They are in some reservoir. They persist in the organism, and whenever a CD19 positive cell comes along, it kills them. So, that can be a problem. There are tricks that are used to carry out bone marrow transplants, which are usually stem cell transplants, to try to genetically edit them. So that the CAR T cells do not recognize the healthy cells but the sick ones do. There are also various other strategies. Then they are very active immunologically, and cytokine release syndrome can occur, for example, which is accompanied by fever and sepsis but actually also intensive care. Various suppressive therapies. Sometimes they also enter the brain causing neurological deficits, neurological damage, so there are various side effects that are not to be trifled with and therefore antigens should be chosen that are specific to the tumor and not in other cells.

Question: Do these side effects occur frequently, or is the risk low for such events?

Answer: Yes, if you administer it systemically, this is the case with many immunotherapies—there are side effects, but it is also a good sign that it is there because then something happens, or you can also manage them, or with various anti-inflammatory therapies, or in the brain, for example, there are also studies of CAR T cells in brain tumors, where you naturally have brain edema or things like that, i.e., swelling of the brain, and you have to make sure that you have strategies to deal with it. But you always learn something new. But it can have potentially fatal side effects.

Question: Regarding the variants of tumors and other diseases such as leukemia, are there also prospects that, for example, cancers like pancreatic cancer could be eliminated because there are not so many really efficient options against pancreatic cancer yet?

Answer: Yes, solid tumors are a hot field at the moment; CAR T cells or I myself am doing this for brain tumors. We are now conducting a study on glioblastoma, which is also an incurable tumor, just like pancreatic cancer. There you always have the problem that you have to get the CAR T cells into the tumor, the so-called homing, and this can be done, for example, by applying the cells directly into the tumor, i.e., not by systemic administration. Or you can give them receptors that facilitate homing into this specific tumor. That is also possible. The problem there is that they are partially switched off or inactivated by the immune system in the tumor. And the third problem is antigen escape. This means that the tumors shut down their antigens and try to hide from the CAR T cells, which is evasionism.

You have to deal with that, and that's why monospecific CAR Ts are probably not the solution for solid tumors.

Question: As a final Question, could you perhaps outline any important findings from your own research or recent cases you've had?

Answer: We are currently working on a CAR T cell product. Very newly generated, and we already have customers. It is used in glioblastomas and targets a tumor-specific target, i.e., EGFRV3, which is only expressed in glioblastomas and not in other body cells, not even in the brain. But only about 30 to 40% of patients have this target, and we have modified these CAR T cells so that they secrete a factor that reprograms the immune system, the macrophages in the tumor. In other words, you have two birds with one stone, so to speak, and these macrophages are then also activated and eat up the other tumor cells that do not have this target. This is a kind of double strategy, and it works wonderfully in mice. All the mice that we treat are actually cured, compared to a normal CAR T cell. And now we want to test that in humans, and then we have to go down this regulatory route. And in the future, we will also try to reprogram macrophages, i.e., macrophages directly, not T cells, but to improve macrophages so that they can also attack the tumor and that they are better directed against the tumor. Because T cells don't actually belong in the brain, we think that microglia or macrophages are a better vehicle for immunotherapy here.

Question: You mentioned earlier that these cells must also be tried in humans. Surely ethics plays a very big role. Are there ways to predict the efficiency of such cells, or must it really just be tried out and then see what happens?

Answer: No, we have already tried to do it with so-called xenograft mice. These are mice in which you initiate human cells that have no immune system, i.e., human cells and glioma cells. And in these systems, you can actually predict quite well that these CAR T cells will work. We have also made cell cultures, i.e., co-cultures of tumor cells directly from humans, i.e., directly from surgical specimens, and have cultivated them with our CAR T cells and see that it works well there, and we know how to sequence the target, which is enough to predict relatively quickly whether it will really work in the corresponding preparations. And therefore, we are quite confident that it will also work in the patients. And these data are then also given to the ethics committee.