CELL & GENE THERAPY INSIGHTS

INTERVIEW

CAR-T cells: from cancer to autoimmunity

Charlotte Barker, Editor, BioInsights, speaks with
Dimitrios Mougiakakos, Professor & MD, Department of Hematology & Oncology, University Hospital Magdeburg

PROFESSOR DIMITRIOS MOUGIAKAKOS, MD has been the director of the Hematology and Oncology department at the Otto-von-Guericke University in Magdeburg, Germany since November 2021. He studied medicine in Hannover and performed his residency at the Universities of Freiburg, Regensburg, and Erlangen, Germany. During his postdoctoral studies at the Karolinska Institute in Stockholm, Sweden he focused on immunotherapeutic concepts against malignancies and against hyper-inflammatory conditions. Moreover, he has a special interest in understanding the metabolic perspective of heterocellular crosstalk and to use these insights for improving immunotherapies. Following his return to Erlangen he was strongly involved in developing and leading the local cell therapy program, where patients with refractory SLE were treated with anti-CD19 CAR-T cells for the first time.

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Dimitrios Mougiakakos joins us to discuss the exciting potential for CAR-T cell therapy for autoimmune disease and how his team approached GMP manufacturing in the clinical setting.

Q How did you first become interested in cell and gene therapy?

DM: As a student, I was very interested in the immune system. I studied medicine at the Medical School of Hanover and my doctoral thesis was on dendritic cells as a key antigen-presenting cell population of the immune system in the context of leukemia.

My interest in immunology continued and as a young hematology resident, I decided to pursue post-doctoral training in Rolf Kiessling's group at Karolinska Institute in Stockholm. Professor Kiessling, the discoverer of natural killer cells, was very much interested in using dendritic cells as a vaccine for cancer patients, so that brought me into contact with cell therapies.

While in Sweden, I also developed a very strong collaboration with Katerina le Blanc, a pioneer in using cell therapy to treat hyper-inflammatory conditions, such as after bone marrow transplantation. Between these two groups, I saw cell therapy both driving and controlling immune responses.

When I returned to Germany and continued my residency at University of Erlangen, I was naturally very interested in continuing to work with cell therapy and went on to launch the clinical chimeric antigen receptor T cell (CAR-T) program at the university's Department of Hematology and Oncology.

Q What led you to your recent work treating system lupus erythematosus ('lupus')?

DM: As a hematologist, I don't typically treat lupus patients. The initial focus of the clinical CAR-T cell program was on patients with lymphoma or other malignant entities – either commercial products or in the experimental setting.

However, my group at Erlangen had a strong connection to the department of rheumatology and immunology. I was a member of several consortia together with colleagues from rheumatology and we often met at the university café to drink coffee, eat snacks, and – most importantly – exchange ideas!

During those discussions, the idea of using CAR-T cells for autoimmune conditions came up. We and many others observed an intriguing off-target effect when treating patients with CAR-T cells targeted at B cell-derived malignancies. As well as killing CD19-expressing tumor cells, the CAR-T cells also kill the non-malignant B cells, which are also CD19-positive. We speculated that this would represent a beneficial effect in autoimmune disorders caused by B cell dysfunction, such as lupus.

Q What is the benefit of using CAR-T cell therapy over monoclonal antibody (mAb) therapies to target abnormal B cells in lupus?

DM: Our colleagues in rheumatology have used mAbs against the CD20 receptor, also found on B cells, in lupus patients, but the results were not as good as they expected. One explanation is that antibodies do not work alone but require other co-factors

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in order to be efficient. When a mAb binds to a target cell, in this case a B cell, it can lead to a direct cytotoxic effect; however, it's more effective to have the complement system co-acting during this time. The abnormal B cells involved in lupus produce autoantibodies that cause constant inflammation. This inflammation is mediated by complement activation, leading to a depletion of the complement factors.

Another problem is the myeloid compartment. When an antibody binds to its target cell it can activate monocyte macrophages that ingest the target cell, B cells in this case. In lupus patients, this phagocytosis can be impaired, and this may also contribute to a lack of efficacious antibodies.

Current evidence suggests that you cannot achieve a full B cell depletion in lupus patients by using mAbs, whereas CAR-T cells can bypass these limitations.

Another problem with previous attempts to control lupus using mAbs might have been the target, with most studies to date utilizing anti-CD20 antibodies. During B cell differentiation from raw B cells to long-lived plasma cells, there are changes in the phenotype. CD20 is already lost at the plasma blast stage. We know that plasma blasts accumulate in lupus patients, and this might indicate that we have not targeted the B cell population that is responsible for producing the harmful autoantibodies. This is speculative but it led us to use CAR-T cells that are not directed against CD20, but rather CD19.

A 2019 study provided support for this hypothesis, showing that CD19-directed CAR-T cells could effectively treat lupus in a mouse model [1].

We hoped that these findings would translate to human patients, with CAR-T cells leading to a deep and long-lasting depletion of B cells and driving an 'immunological reset'.

Q What have been the results of the human trials so far?

DM: In an early stage of our discussions, our colleagues in rheumatology mentioned a female patient in her early 20s, whose lupus was having a profound impact on her life. Like many people, I didn't know a great deal about lupus. I learned that in Europe there is an incidence of 100 cases per 100000 people, so we expect 300000 patients per year. Lupus can affect all organs in the body and can dramatically affect quality of life, requiring ongoing treatment. There is no standard of care for patients with refractory disease so there is a high unmet clinical need in patients that do not respond to the state of the art.

The young woman being treated in the rheumatology department had several organs affected, including her kidneys, heart, and lungs. Our colleagues had exhausted all treatment options, but her disease remained refractory, leaving her unable to attend school or go about her regular life as she had before the onset of the disease. We discussed the possibility of CAR-T therapy with this patient and her close relatives, and she told us she would like to go ahead.

Uncertain whether it would work, we set about producing the CAR-T cells. The first challenge was efficiently collecting the T cells from the patient. Patients with lupus normally have low T-cell numbers and have received several agents that can negatively affect T-cell function so we were unsure whether the cells would function correctly. But we collected enough T-cells,

transduced them *in vitro*, expanded those cells, and were able to transfuse one million fresh CAR-T cells per kilogram of body weight. To our delight, it worked!

Toxicity levels were very low, with no severe signs of the most common CAR-T cell-associated toxicities – cytokine-release syndrome or neurotoxicity. We saw a strong expansion of the CAR-T cells within the patient's own body. By day nine she reached peak expansion, with almost a third of all of her T cells being CAR-T cells.

Within three weeks, all autoantibodies were at zero, kidney function was improved and complement concentrations were back to normal. One year after CAR-T cell treatment, the patient is off medication, has no symptoms, and is effectively back to normal. CAR-T cells are still detectable in peripheral blood and for this patient, it appears that we have achieved the immunological reset we hoped for.

We have now treated six patients. The data are now being evaluated for publication and are very encouraging. The good efficacy of CAR-T cell therapy in patients with lupus is also encouraging in terms of other autoimmune disorders.

For any physician, it is our greatest achievement to see our patients doing better. Just last Thursday we had a get-together with the first few patients and treating physicians and it was wonderful to see how the patients benefited from this treatment and how happy they are with the results.

Q What were the operational challenges of setting up cell therapy in your clinical setting?

DM: The use of approved commercial cell therapy products is already very demanding, involving various certification processes to make sure that the medical team (administration, nurses, physicians) is fit to carry out such treatment and that the structure of your hospital can keep up with the requirements for cell therapy – the whole hospital structure is under evaluation.

When you want to produce CAR-T cells within your setting, there is another level of challenge. In terms of regulatory requirements, it is a huge thing to carry out because you are producing three things in one: an immunotherapy, a cell therapy, and a gene therapy.

And there are different requirements set up by the regulatory organizations for all those three therapeutic forms, and CAR-T cell producers must fulfill them all. This requires a lot of time and investment, a highly specialized team with experts in good manufacturing practice and good clinical practice, and the appropriate facilities. We were very lucky to

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have the support of the university and the university hospital in Erlangen to set up our own CAR-T cell unit.

Q How did advances in technologies help you overcome those challenges?

DM: Technology is developing rapidly in this area. We used the CliniMACS Prodigy® (Miltenyi Biotec) instrument, which allowed us to carry out a semi-automatic manufacturing process, with the device taking care of culturing and expanding the cells. This has several advantages. First, the process is very standardized. Second, it removes people from the bench who would otherwise be required to carry out the culturing, so we can reallocate our staff towards quality management and facility management of clean rooms. That was beneficial for the whole program because it is not only more efficient as an organizational structure, but also reduces the user-dependence of the manufacturing process.

We also benefited greatly from switching from generating the cell product on the bench to generating it within a closed system.

Q Do we need decentralized CAR-T cell production in the academic setting?

DM: It is a considerable investment; however, I believe it's important. Of course, I don't think that a decentralized CAR-T cell production will produce high-throughput cell products, like a company facility.

However, industry and academia have different perspectives, and having the two sides work together is a big advantage. Academia can do things that no company would have done. When you treat patients every day, and you have your own facility next to you, many ideas come up via this close interaction.

Q What challenges remain? Where do you see technology could make an impact?

DM: Challenges remain in three key aspects of cell therapies, particularly with CAR-T cell therapy – efficacy, timing, and safety.

Safety does not appear to be of major concern, at least in the fields where we have utilized CAR-T cells to date. I believe we can treat our patients in a very good and standardized fashion. People throughout the world have done a great job interacting and exchanging experiences to establish protocols that are beneficial for our patients. In my own experience using CAR-T cell therapies, we can manage most of the side effects. However, it's still possible to make it even safer; for example, there are developments like constructs with off-switches that allow us to deactivate CAR-T cells.

Timing is an important factor, especially when treating patients with malignant disease that is refractory or resistant to conventional therapy. Collecting the cells and generating the CAR-T

cell product can take two to three weeks. During this time, the tumor continues growing and causing problems, and we sometimes lose patients before they can receive the treatment.

There are different strategies in order to address timing. Studies have been carried out using allogeneic CAR-T cells, which can be matched to the patient, or genetically modified. Another possibility is to speed up the production time. A recent paper described generating expanded CAR-T cells in 48 h, which is amazing. A combination of those approaches will help us to treat more patients in a timely fashion.

Then we have the issue of efficacy. CAR-T cell therapy works in many malignant diseases, but it doesn't work 100% and we need to understand why. We need to understand how the disease escapes the CAR-T cell therapy, for example in solid tumors.

CAR-T cells do not work as well in solid tumors as they do in lymphoma and leukemia. There are various strategies to address this problem; one very interesting way is to reactivate CAR-T cells within a patient. This is based on evidence that in trying to enter the dense tissue of a solid tumor, a CAR-T cell 'forgets' its target, becoming dormant. You can reactivate it by vaccinating the patient with the target antigen, helping the CAR-T cell remember the target and continue its journey through this hostile microenvironment. These strategies are currently being evaluated within clinical trials and I believe we are on the right track to increasing efficacy.

Diseases outside of cancer bring other challenges. The immune system is very pleiotropic and has many functions, being involved in all homeostatic processes. Therefore, I think cellular therapies could be of use in many diseases, including autoimmune conditions and fibrosis. There have been several murine models evaluated that support the notion that CAR-T cell therapies will not be limited to the field of cancer treatment.

Q In which areas do you see the brightest future for cell and gene therapy?

DM: The field has changed the way we treat cancer patients and there is still huge potential to increase efficacy and apply CAR-T cells in a plethora of new indications.

Based on my personal experience and our observations using CAR-T cells in lupus, I think that autoimmune disorders could be the 'next big thing' in CAR-T cell treatment. We have seen amazing outcomes in our patients, exceeding the response to existing therapies.

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