

Final report

Leibniz Transfer

Selective depletion of plasma cells secreting pathogenic autoantibodies in chronic autoimmune diseases – a potentially curative therapeutic approach (PCdepCAD)

Project number: T85/2020

German Rheumatology Research Center (DRFZ), An Institute of the
Leibniz Association

Project duration: July 2021- 30 June 2025

Project leader: Prof. Dr. Falk Hiepe

Executive Summary

This transfer project is a continuation of research conducted at the German Rheumatology Research Center (DRFZ) on the role of plasma cells in autoimmune diseases. The evidence provided by the DRFZ that autoantibodies can be produced by long-lived plasma cells that are resistant to conventional immunosuppressive therapies has contributed significantly to plasma cells becoming increasingly important as a therapeutic target, particularly in refractory autoimmune diseases. However, the plasma cell therapies used to date lead to the indiscriminate ablation of all plasma cells, including those that produce protective antibodies, which increases the risk of infection. A therapeutic approach developed and patented at the DRFZ, on the other hand, targets the antigen-specific ablation of only those plasma cells involved in the pathogenesis of the disease. This is achieved with a bispecific conjugate consisting of an antibody directed against plasma cells (anti-CD38 or anti-CD138) and the (auto)antigen coupled to it. After injection of the conjugate, all plasma cells are marked with the (auto)antigen. Plasma cells that secrete autoantibodies against the labelled autoantigen immediately bind to the secreting cell, which is driven to suicide via complement- or antibody-mediated cytotoxicity. That is why we refer to this conjugate as an “antibody-mediated cytotoxicity engager” (ACE). In our PCdepCAD project, this therapeutic approach was investigated for the first time in models of autoimmune diseases such as myasthenia gravis and systemic lupus erythematosus. In addition to an already established mouse model (experimental autoimmune myasthenia gravis, EAMG), which induces the disease through immunization with the acetylcholine receptor (AChR), a humanized mouse model of myasthenia gravis was established to test human AChR-ACE products. NZB/W mice, which spontaneously develop anti-dsDNA autoantibodies and nephritis, were used as a lupus model. The ACE used in the models differ in terms of the autoantigens and antibodies used: murine α -ECD (alpha subunit of the extracellular domain of the acetylcholine receptor (AChR), which contains the main immunogenicity region) and anti-mouse CD138 in EAMG; human α -ECD, anti-human CD38 in the humanized model of myasthenia gravis, anti-mouse CD138 and a 100 base pair long dsDNA oligomer (dsDNA-ACE) in the mouse model of lupus nephritis. The Fc portion of the antibody must be modified in such a way that they cannot perform effector functions that cause cell death, among other things. The ACEs were produced in collaboration with our partners at FMP (Hackenberger), Hellenic Pasteur Institute Athens (Lazarides), and Sanofi-Aventis (Martinez-Cingolani). To establish the humanized mouse model of myasthenia, mouse hybridoma cell lines that secrete autoantibodies against AChR were transfected with human

CD38. For the first time, we were able to demonstrate in both the humanized mouse model of myasthenia and the lupus model that the AChR- and dsDNA-specific plasma cells could be eliminated highly selectively and effectively after injection of the corresponding ACE, preventing the development of the respective disease.

During the four-year project period, which was extended due to restrictions imposed by the COVID pandemic, it was possible to complete most of the key work packages and milestones outlined in the project proposal, which were adjusted based on the research findings. The results have been/will be published in high-ranking journals. Two patents were filed during the application period. The novel therapeutic principle was presented several times at high-profile international meetings. The process also attracted the interest of investors and led to the spin-off of Methuselah Bio and Aluma Therapeutics, Inc.. Aluma Therapeutics and the DRFZ have entered into a collaboration agreement with the aim of developing plasma cell-targeted therapies for autoimmune diseases. With the help of financial resources of the project, two postdoctoral researchers were able to lay the foundation for their dissertations.

1. Contribution to strategic goals of the funding programme

Chronic autoimmune diseases cause a permanent high social and economic burden on the healthcare system. This is due to the cost of medication and indirect costs arising from side effects of drugs, comorbidities such as infections, cardiovascular and cerebrovascular diseases due to chronic use of steroids and immunosuppressants, and sick leave, which also has social implications and affects patients' quality of life.

The successful proof-of-concept studies in two autoimmune mouse models represent an important step toward translating this innovative therapeutic strategy with curative potential into patient care, which could improve quality of life and significantly reduce direct and indirect costs.

2. Achievement of objectives and milestones

WP 1: Selective depletion of Autoantigen-specific plasma cells using autoantigen-anti-CD138 conjugate in experimental autoimmune myasthenia gravis (EAMG) and mouse model of lupus nephritis

Partners: Meisel, Hackenberger, Lazaridis

To reproduce an EAMG in C57BL/6 mice and study the therapeutic effect of a mouse bispecific conjugate (mouse AChR coupled to mouse anti-CD138 antibody)

Implementation

Reproduce EAMG: 100% completed

Production of affinity matrix: 100% completed

Treatment of EAMG: 100% completed

WP 2: Selective depletion of AChR-specific plasma cells using hAChR-anti-CD38 conjugate (hACR-ACE) in human myasthenia gravis

to produce a human bispecific conjugate consisting of an α -subunit of the extracellular domain of the human acetylcholine receptor (AChR) and an anti-human CD38 antibody with a non-functional Fc portion and test it in a humanized mouse model of myasthenia.

Partners: Hackenberger, Meisel, Rückert, Lazaridis, Martinez Cingolani, Kruglov

Implementation:

Generation of human AChR (and anti-huCD38 antibody: 100% completed

Production of affinity matrix: 100% completed

Set up ex vivo / in vitro system: 100% completed

Depletion of AChR-specific plasma cells ex vivo: 100% completed

Establishment of a humanized mouse model: 100% completed

Treatment of a humanized mouse model: 100% completed

WP 3: Preparation of an investigator-initiated phase 1/2 trial in active MG patients despite standard of care treatment

This WP was discontinued because, despite intensive efforts, we and our cooperation partners were unable to manufacture a product suitable for patients.

3. Activities and obstacles

The COVID-19 pandemic has led to several interruptions in scientific activities in the project in 2021 and 2022. This had a significant impact on the recruitment of scientific staff, laboratory experiments, general activities, and the collection of patient samples.

Unexpected difficulties arose in establishing the bispecific conjugate that we now name “antibody-mediated cytotoxicity engager” (ACE), which consists of the autoantigen and an antibody that labels the plasma cells on their surface with the autoantigen. This is due to the structure of the autoantigen, the alpha subunit of the extracellular domain of the acetylcholine receptor (α -ECD). To solve the problem, we started a collaboration with the research group of Dr. Kostas Lazarides from the Hellenic Pasteur Institute in Athens (Greece), which has extensive experience in the recombinant production of autoantigens relevant to myasthenia. We pursued three different approaches to establish the AChR-ACE: 1. chemical coupling of the autoantigen to an antibody that recognizes plasma cells, with our partner at the FMP, 2. production of a recombinant product with the support of Dr. Lazarides' research group and Sanofi, and 3. coupling of the biotinylated autoantigen to the antibody via streptavidin. Despite intensive efforts in collaboration with the FMP, no satisfactory solution could be found for the chemical coupling of α -ECD of the AChR to the antibody. The production of recombinant AChR-ACEs was successful. However, only small quantities could be produced, which were only sufficient for in vitro experiments. Therefore, AChR-ACEs produced using the biotin-streptavidin interaction were used for the therapeutic experiments. However, since this product cannot be tested on humans, WP 3 was not continued. The funds earmarked for this purpose were therefore reallocated.

In deviation from the original project proposal, we also investigated another autoimmune model in **WP1** that develops spontaneously, in contrast to EAMG. This is the NZB/W mouse, which suffers from lupus nephritis due to the production of anti-dsDNA autoantibodies. Our group has many years of experience with this model. We produced a dsDNA-ACE by coupling 100 base pair long dsDNA oligonucleotides, which we identified as suitable target for anti-DsDNA antibodies to a mouse anti-CD138 antibody. To avoid effector functions, the antibody contained a non-functional human Fc portion. Administration of the dsDNA-ACE at two-week intervals led to a significant reduction in anti-dsDNA antibodies and halted the development of nephritis. After 10 weeks, we discontinued therapy due to the formation of antibodies against the human Fc portion and streptavidin. For future studies with this animal model, we produced a mouse anti-CD138 antibody with a non-functional mouse Fc portion and successfully coupled the benzoacryl-labeled 100-bp dsDNA oligonucleotide to accessible cysteine residues of the antibody.

We had to solve another problem when establishing the humanized mouse model of myasthenia. The original plan was to adoptively transfer AChR-specific plasma cells from the thymus of myasthenia patients after thymectomy into immunodeficient mice as described in the literature. However, the number of AChR-specific plasma cells obtained was very low, so we could not assume that their transfer would trigger a disease. Experiments to increase the number of plasma cells by stimulating B cells ex vivo also failed. We therefore decided to transfer hybridoma cell lines that produce human anti-AChR autoantibodies to immunodeficient mice. However, since these were mouse cells, human CD38, the target of the AChR-ACE, was expressed on the surface of the cells by means of transfection with the help of A. Kuglov's group at the DRFZ and MDCell at the Max Delbrück Center.

4. Results and successes

Results of the project have led to 3 publications. In Khodadadi et al. (Eur. J. Immunol. 2026, in press), we were able to show that selective depletion of pathogenic plasma cells that secrete autoantibodies against the AChR by an antibody-mediated cytotoxicity engager (ACE), consisting of an anti-human CD38 antibody conjugated to the α ECD of the AChR, led to lower autoantibody titers and prevented disease progression in a new humanized mouse model for myasthenia gravis established in this project.

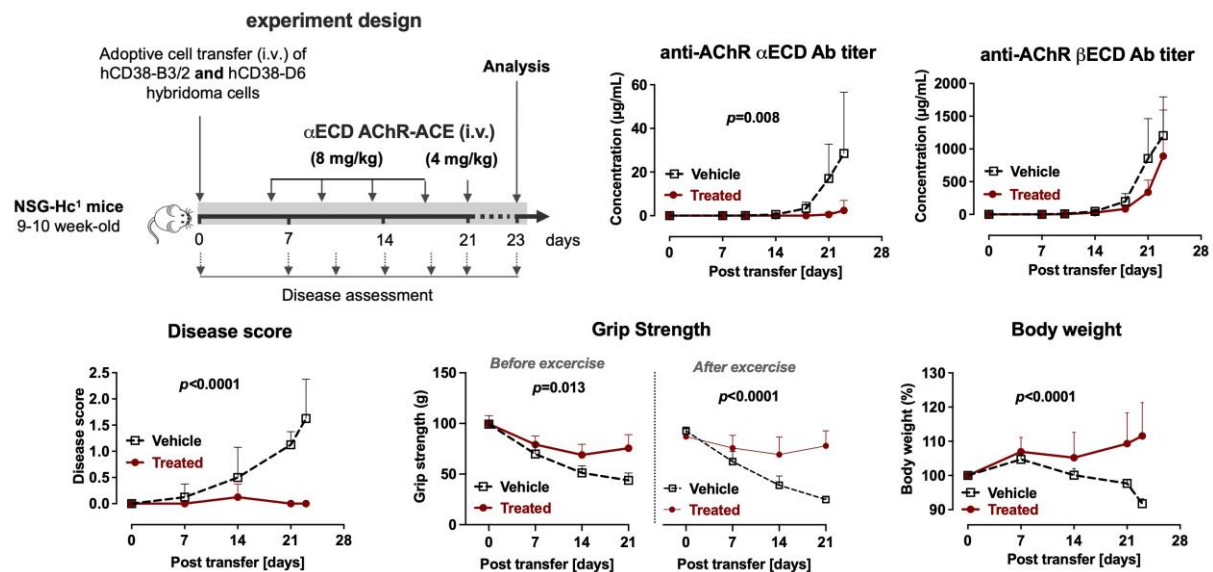


Figure 1: Treatment with α ECD AChR-ACE prevents the production of pathogenic anti- α ECD antibodies and development of disease in a humanized model of myasthenia

In Cheng et al. (Proc Natl Acad Sci USA, 2026, under revision), we show in a mouse model for lupus, characterized by the spontaneous development of nephritis due to the production of anti-dsDNA autoantibodies, that selective depletion of dsDNA-specific plasma cells using a dsDNA-ACE consisting of a mouse anti-CD138 antibody conjugated to a 100 base pair dsDNA oligonucleotide improves the development of nephritis. Other plasma cells were not affected by dsDNA-ACE.

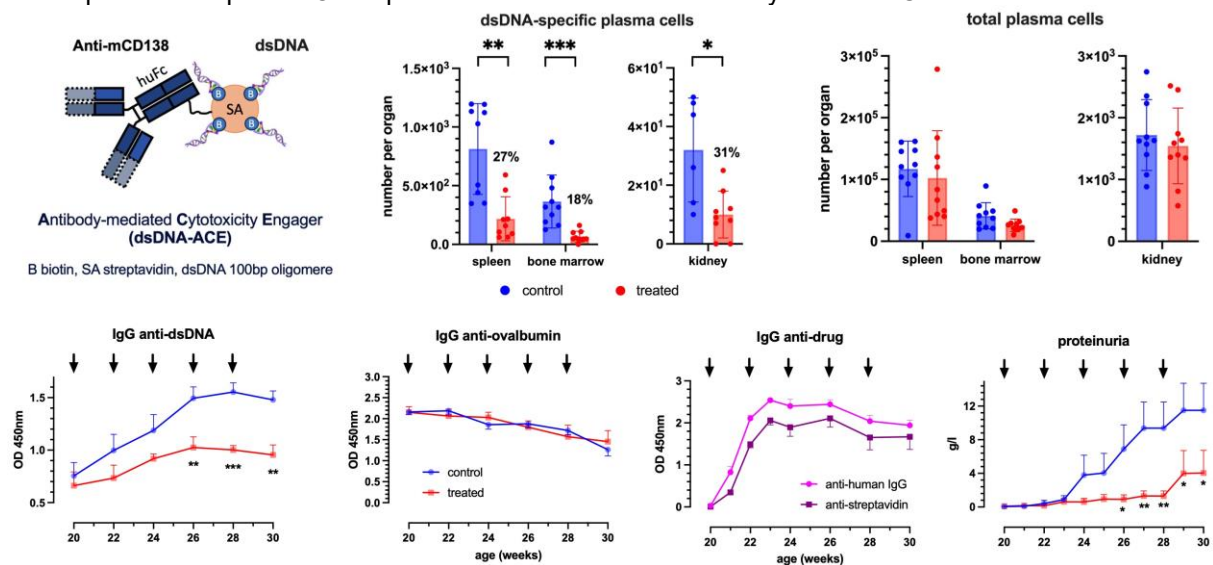


Figure 2: Treatment of lupus mice with dsDNA-ACE results in a significant ablation dsDNA-specific plasma cells 48 hours after its administration (upper row). Continuous therapy with dsDNA-ACE every 2 weeks selectively inhibits the formation of anti-dsDNA antibodies and stops the development of proteinuria (bottom row). The arrows represent the timing of dsDNA-ACE administration.

In both autoimmune models, we were thus able to show for the first time that highly selective depletion of pathogenic plasma cells can halt the disease.

In preparation for establishing a humanized mouse model of myasthenia gravis, we examined 24 myasthenia gravis patients who underwent thymectomy for anti-AChR antibody titers in serum and AChR-specific B and plasma cells in peripheral blood and thymus samples using flow cytometry at the time of surgery. AChR-specific plasma cells or their precursor B cells were identified in the thymus derived from all 13 myasthenia gravis patients with high anti-AChR antibody levels as well as in 2 of 3 patients with low anti-AChR antibody levels regardless of the onset of the disease. In contrast, no AChR-specific plasma cells or B cells could be detected in any of the 8 seronegative patients. Our findings highlight comparable frequencies of AChR-specific B and plasma cells in both early onset and late onset myasthenia gravis patients, underlining the therapeutic potential of targeting autoreactive cells in both groups of patients. Importantly, this result has also implications for the still controversial question of

whether thymectomy can be beneficial not only for early onset but also for late onset myasthenia patients (Brain Communication, submitted).

Two patents were filed during the application period. The novel therapeutic principle was presented several times at high-profile international meetings. The process also attracted the interest of investors and led to the spin-offs Methuselah Bio and Aluma Therapeutics, Inc.. Aluma Therapeutics and the DRFZ have entered into a collaboration agreement with the aim of developing plasma cell-targeted therapies for autoimmune diseases. With the help of financial resources of the project, two postdoctoral researchers were able to lay the foundation for their dissertations.

5. Equal opportunities, career development and internationalisation

The DRFZ and the institutions involved in the project are committed to equal opportunities. When advertising job vacancies, women with the same qualifications are given preference. The DRFZ has been awarded the TOTAL E-QUALITY rating. The project team at the DRFZ consisted of the project manager, two postdocs (one male for the entire project period and one female for one year) and two female PhD students. The two PhD students will complete and submit their dissertations in 2026.

6. Structures and collaboration

Our partner in the Department of Chemical Biology at FMP Berlin worked on solutions for the successful chemical coupling of the autoantigen to antibodies that recognize plasma cells in order to develop a soluble, standardized affinity matrix product for long-term use.

We worked closely with our partners at Charité (neurology, surgery) on the in-depth immunological characterization of patients with myasthenia gravis. Thymus material and blood from 37 thymectomized myasthenia patients were made available to us for further characterization. This was particularly important for the establishment of a human myasthenia mouse model.

A very important collaboration was established with the research group of Konstantinos Lazaridis (Laboratory of Immunology, Hellenic Pasteur Institute, Athens, Greece). This research group is considered an internationally leading group dealing with the immunopathogenesis and therapy of myasthenia gravis. Based on their experience in the recombinant production of autoantigens relevant to myasthenia, such as the acetylcholine receptor, they supported us in establishing suitable recombinant AChR molecules for the AChR-ACE.

We collaborated with MDCell at the Max Delbrück Center to express human CD38 on murine hybridoma cells that secrete pathogenic anti-human α -ECD autoantibodies via transfection. Using these cells, we were able to test the efficacy of a human α -ECD AChR-ACE in their depletion. We were also able to show that the transfer of these cells into immunodeficient NSG mice induces myasthenia with high anti- α -ECD autoantibody levels, which we used as a model for therapeutic in vivo experiments with a human AChR-ACE consisting of an α -ECD-AChR and an anti-human CD38 antibody conjugate.

7. Quality assurance

Animal experiments are being conducted as part of the project. All animal experiments were conducted in accordance with German animal welfare regulations and the 3R principles, with ethical constraints limiting animal numbers and experimental scope. Although this may reduce statistical power, it reflects a balance between scientific rigor and ethical responsibility. Weekly research group meetings are held to discuss the latest results and the next experiments. The results were also regularly presented and discussed at the weekly meeting of the B-Cell Club (where all researchers interested in B/plasma cell projects meet) and the DRFZ Institute Seminar. The publications were submitted to specialist journals that subject scientific content to a rigorous peer review process.

8. Outlook

Building on the results achieved in these proof-of-concept studies, which demonstrate for the first time in autoimmune models the therapeutic efficacy of selective ablation of pathogenic plasma cells, we will continue our scientific work to optimize this therapeutic principle. This includes establishing suitable methods for chemically coupling autoantigens to antibodies, which is a prerequisite for ACE therapy in patients, as well as the targeted ablation of related precursor B cells to prevent the repopulation of pathogenic plasma cells. To this end, we will continue our collaborations with the FMP and the Hellenic Institute in Athens. Corresponding research proposals, including to the German Research Foundation (DFG), are currently being prepared.

In close collaboration with DRFZ, Aluma Therapeutics is developing a plasma cell-targeted therapy for an autoimmune disease for which there are currently few therapeutic alternatives.