Deep immune monitoring of chronic GvHD patients treated with polyclonal Treg therapy

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established curative option for various hematological diseases. However, chronic graft-versus-host disease (cGvHD) affects every second transplanted recipient, presenting a significant challenge. Immune-modulatory cell therapy, specifically regulatory T cell (Treg) therapy, is a promising solution to reduce reliance on conventional immunosuppressive drugs.

In collaboration with the CRTD GMP facility, Anke Fuchs and Martin Bornhäuser from Medical Clinic I, Universitätsklinikum Carl Gustav Carus Dresden, treated 10 steroid-refractory cGvHD patients using polyclonal Tregs within a named patient program. To evaluate the persistence of adoptively transferred Tregs and monitor changes in the peripheral immune system linked to clinical efficacy, serum and PBMCs were collected at various time points before and after Treg therapy.

In this study, we employ deep immune monitoring techniques, including Cytometry by time-of-flight (CyTOF). A 48-parameter, 39-marker CyTOF panel was designed for PBMC analysis, encompassing markers for barcoding, cell ID, viability, activation, and immune response. The panel's establishment and validation utilized PBMC samples from three healthy donors, identifying major immune subsets through a comprehensive gating strategy. For result reproducibility and comparability, anchor samples were prepared, as patient samples will be divided and analyzed in different batches. Additionally, we are developing a data analysis pipeline using a combination of R software and OMIQ software. The CRTD CyTOF facility's acquisition of the advanced mass cytometry machine, the CyTOF XT, is anticipated to reduce cell loss during sample acquisition. Comparative experiments between the new CyTOF XT and the older CyTOF2 instrument have been conducted to assess performance.

The outcomes of these studies aim to define immune markers of clinical efficacy, potentially predicting individual responses and treatment outcomes. An enhanced understanding of the mode of action of adoptively transferred Treg cells will contribute to the development of advanced suppressive cellular therapies, aligning with the primary goals of our lab.