DESIGN AND OPTIMIZATION OF MASS CYTOMETRY TO MEASURE IMMUNE RECONSTITUTION POST-HEMATOPOEITC STEM CELL TRANSPLANT

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Hematopoietic stem cell transplant (HSCT) is an effective cancer immunotherapy treatment for patients with hematological malignancies. While HSCT is a curative treatment, patients are at high risk of developing graft-versus-host disease (GVHD). Understanding immune reconstitution post-HSCT is important for discerning the mechanisms controlling the desired graft-versus-leukemia effect versus GVHD, as well as understanding the mechanisms of action of common immunosuppressive therapies used in HSCT, such as anti-thymocyte globulin (ATG). Immune reconstitution is a complex process involving multiple cell types and subsets, making it challenging to measure in limiting samples from lymphopenic patients. We aimed to develop a new mass cytometry-based method to measure expression of up to 40 parameters measured simultaneously in one million PBMCs, creating a comprehensive immune reconstitution panel that would not be possible using conventional fluorescence flow cytometry. Because reconstitution of regulatory T cells (Tregs) has a major impact on transplant outcomes, we first focused on developing a novel protocol that enables simultaneous detection of FOXP3, the Treg lineage-defining transcription factor, cell surface markers and cytokines on cryopreserved PBMCs. We first tested the conventional protocol to detect FOXP3, which is optimized for fluorescence flow cytometry, and found that it failed to detect FOXP3 by mass cytometry. We therefore tested a variety of different methods to fix and permeabilize cells, and then created a new protocol that allows robust and sensitive detection of FOXP3. This new protocol was also more sensitive than commercially-available protocols optimized for mass cytometry. We also compared different anti-FOXP3 mAbs and found that the 236A/E7 clone was the most sensitive. With this optimized method, we are now able to measure expression of FOXP3 together with 36 other proteins and experiments to measure how immune reconstitution is affected by ATG in patients who have undergone a hematopoietic stem cell transplant.

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