

Harmonized T cell monitoring assays as biomarkers for immunotherapy trials: A data driven approach by the CIMT Immunoguiding Program ("CIP")

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Abstract

Measurement of the quantity and quality of vaccine-induced responses is applied by many investigators to gain insight into *in vivo* effects of T-cell based immunotherapies and optimize their vaccination approaches. T cell immunomonitoring techniques also bear the promise to present surrogate markers for clinical endpoints in the future. Only validated assays which guarantee robust and reproducible results should be used for guidance of clinical development. Although international rules exist that describe how biomarker assays should be validated in theory, there is a lack of practical information on how to proceed with the validation of commonly used T-cell based immunomonitoring assays. In the last few years, international initiatives have been launched to optimize and validate immunomonitoring assays, contributing to an increased interest for assay validation among the immunotherapy field.

The "CIMT Immunoguiding Program" is an international working group founded under the aegis of the Association for Immunotherapy of Cancer (www.c-imt.org). It includes twenty four laboratories from eight European countries, and aims at optimizing protocols and validating techniques used for monitoring of antigen-specific T cells. The group has already reported on two consecutive testing phases where HLA-tetramer staining and IFN- γ ELISPOT were tested for detecting antigen-specific CD8+ T cells. For this, pre-selected PBMC samples and relevant reagents (synthetic peptides and HLA-multimers) were prepared centrally and distributed, the frequencies of peptide (virus)-specific cells were determined individually, and the sensitivity of the assays was then compared among participants. Results from the first phase were used to deduce technical recommendations for both assays and the experiments were repeated in a second phase, using optimized protocols. This two-step approach could formally show that correction of the protocols successfully increased the performance and reduced the inter-center variability of the assays. The assay recommendations are available at the homepage of the group. Two new testing phases have been recently started, for HLA-tetramer staining and IFN- γ ELISPOT, focusing on selected protocol variables which are hypothesized to influence the assay performance. Additionally, intra-cellular

cytokine staining has been addressed in a third independent panel.

In conclusion, participation in proficiency panels such as the CIMT Immunoguiding Program can offer several advantages. First, they propose external validation to participating labs on a regular basis, providing direct feed-back on the quality of locally established protocols. Second, they identify parameters responsible for lack of sensitivity and for intra-laboratory variation, leading to protocol improvement. Finally, they constitute a rational basis for harmonization of immunomonitoring techniques which could accelerate the development of new therapies to the benefit of patients.