Abstract
The Okada lab recently implemented a vaccine study in which WHO Grade II glioma patients with pre-surgical administration of immunotherapy followed by surgical resection and evaluation of the resected tumor. This study is evaluating whether varilumab, a monoclonal antibody against CD27, enhances induction of CTL responses against 10 glioma-derived T-cell epitopes included in IMA950 vaccine in patients’ peripheral blood and tumors.

Due to heterogeneity in solid cancers, targeting a single antigen may result in selection of subclones that lack the target antigen. While this clinical trial addresses the issue of antigen heterogeneity by targeting 10 IMA950 epitopes, the main challenge evaluating T-cell responses mounted against the 10 epitopes is limited amounts of tumor and blood samples. CyTOF mass cytometry is a newly emerging technology that allows for monitoring of multiple immune subsets from small tumor samples, making it ideal for this clinical trial. In 2017 I successfully created mass cytometry-compatible HLA-tetramers for IMA950 antigens and validated these metal-labeled HLA-tetramers using human T-cells stimulated with IMA950 peptides in vitro.

Successful completion validated the performance of these novel tetramers by mass cytometry and justifies their use on more precious glioma patient samples with a larger set of T cell phenotyping antibodies. In 2018, the objective will be to detect increases/changes of IMA-950 reactive T-cells in pre- vs. post-treatment samples.

Methods
CyTOF mass cytometry is a cutting edge technology and an emerging single-cell analysis platform that uses atomic mass spectrometric analysis to quantify up to 42 metal-tagged antibodies per cell.1 The large number of analytes per cell allows the simultaneous monitoring of multiple immune subsets from small tumor samples, making it ideal for this clinical trial. CyTOF has recently been utilized to study the immune microenvironment of resected human gliomas. Fluorescence-labeled HLA-peptide tetramers have been widely used for assessments of epitope-specific T-cell responses. Mass cytometry measurement of HLA tetramers has been previously used to compared immunogenic viral epitopes in human clinical samples.2

Rationale
Analysis of immune cells and their functional states is fundamental to understanding the pathogenesis of glioma. Currently, immunologists utilize fluorescence cytometry to analyze up to 8 markers within a sample, but the major limitation is the overlap of emission spectra of fluorescent antibody labels. As previously described CyTOF utilizes nearly 40 labels within a sample and the distinct mass resolution eliminates the limitation of overlap of various channels.

Aim 1. Creation of mass cytometry-compatible HLA-tetramers for IMA950 antigens
Aim 2. Validation of the metal-labeled HLA-tetramers using human T-cells stimulated with IMA950 peptides in vitro
Aim 3. Pilot evaluation of IMA950-reactive T-cells in tumor and blood samples obtained from the neoadjuvant vaccine study

Results

![Unstained Jurkats H3.3 TCR transduced](image1)

![Jurkats H3.3 TCR transduced- NA-DL650](image2)

![Jurkats H3.3 TCR transduced- SA-DL650](image3)

![Jurkats H3.3 TCR transduced- H3.3 Tetramer BL (2 µl)](image4)

![Jurkats H3.3 TCR transduced- H3.3 Tetramer BL (4 µl)](image5)

![Pacific Blue-A](image6)

![Unstained Jurkats H3.3 TCR transduced](image7)

![Jurkats H3.3 TCR transduced- H3.3 Tetramer BL](image8)

![Pacific Blue-A](image9)

Significance
Successful completion of the proposed study will establish mass cytometry-based tetramer assays as a method to simultaneously evaluate T cell phenotype, functional status, and antigen-specific reactivities. This will serve as a powerful tool to learn about how the anti-tumor immune response could be activated and the impact of the tumor microenvironment on the functional status of vaccine-induced T-cells both systemically and locally.

Future Directions
Using available PBMC and TIL samples, the goal is to evaluate whether mass cytometry-based tetramer analyses will detect induction of IMA950-reactive T-cell responses using the method described in Aim 2. The Okada lab had proposed IFN-γ enzyme-linked immuno-spot (ELISPOT) assay as the primary assay method to evaluate IM950-reactive T-cell responses. Therefore, we will compare the results obtained from ELISPOT and tetramer assays. If the regimen induces IM950-reactive T-cell responses, we should be able to detect responses in both types of assays.

Conclusion
This study entailed developing mass cytometry probes in order to investigate T-cell diversity. We utilized molecular biology and cancer immunotherapy techniques to create HLA tetramers using a conjugation procedure. We successfully created tetramers for three IMA950 antigens, FABP7, PTP-005, and TNC-001. Our preliminary results of fluorescent tetramer staining validated the performance of these novel tetramers compared to a commercial fluorescent tetramer which served as a positive control measure.

References

Thanks to Dr. Hideho Okada and Dr. Payal Watchmaker, Department of Neurosurgery, UCSF for support and guidance.

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