CARTography: Mass Cytometry Based Approach to Deep Phenotyping of Anti-CD19 CART Cells

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Purpose

Adoptive cell based immunotherapy is a novel approach that enables specific clearance of malignant cells expressing tumor-associated antigens. In anti-CD19 CART cell therapy (CTL019), a patient's own T cells are genetically engineered to express a chimeric antigen receptor (CAR) which recognizes and kills CD19 positive leukemic cells. Due to the intrinsic heterogeneity of T cells, it is critical to map and comprehend the diversity of T cell phenotypes during the manufacturing of CART cell product.

Methods

Time of flight mass cytometry (CyTOF®) enables generation of highly multiplexed immune cell panels which empower scientists to quantify the phenotypic landscape of CTL019 immunotherapy cell products at the single cell level. We have designed a custom 37 parameters CyTOF T cell biomarker panel which include classical cluster of differentiation (CD) markers to identify T cell lineage, subset, phenotype, activation status, as well as expression of immune-activating and immune-inhibitory receptors. Cryopreserved CART expressing cells were stained using custom 33 antibody mix following Fluidigm® cell surface staining protocol. Data acquisition and normalization was completed according to manufacturer's recommendations. Analysis was conducted using various clustering algorithms as well as classical gating approach in order to quantify cell population frequencies and biomarker expression intensities. Subsequent data mining for biomarker discovery was conducted to identify differential biomarker expression.

Results

This publication focuses on case studies highlighting approaches to CyTOF® data dimensionality reduction, complex cytometry data mining and results interpretation. In this study we have employed multiple methods of single cell data dimensionality reduction which are commercially available in Cytobank®. First step of CyTOF data dimensionality reduction resulted in automated sorting of randomly acquired cells into structured groups of cell subsets with similar marker expression. These groups of sorted cells, or clusters, were then compared for frequency and their associated phenotype. This approach was sufficient in the initial deconvolution and worked well with interpreting results from few samples, however, second level of complexity reduction was needed to identify cellular biomarker patterns between large groups of samples containing complex metadata information on experimental condition and outcomes. Methods employed in this analysis include hierarchical clustering, principal component analysis (PCA) of clusters of cells as well as ANOVA. This statistical analysis approach to CyTOF data analysis resulted in identification of differences in cell frequencies between groups of experimental samples. Targeted data mining allowed creation of a short list of cellular biomarkers differentially expressed between groups of healthy donor samples (n=14) undergoing alternate manufacturing processes. Phenotypic differences between CD4 and CD8 cells (n=7, $p \geq 0.05$) were also identified and mapped back to corresponding functional CD biomarkers.

Conclusion

We have generated highly multiplexed CyTOF data on cellular therapy product, through data analysis and mining approaches we have successfully characterized T cell subset frequencies and uncovered their corresponding phenotypes during different stages of various manufacturing processes. Deep characterization of immune cells through mass cytometry approach provides a powerful tool for decoding the complexity of immune cell compartments and cellular biomarker discovery. Specific T cell phenotypes, or associated map locations, can then be used to correlate product phenotype with cell manufacturing process, patient outcomes and/or safety profiles.