Characterizing CAR T Cell Therapy Biomarkers Through Multianalyte Analysis

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Introduction

Identification and monitoring of biomarkers related to T cell activation and associated cytokine release syndrome (CRS) will be necessary to fully realize the immense potential of chimeric antigen receptor (CAR) T cell therapy. Such biomarkers could be used to guide clinical development of candidate therapies[1], provide mechanistic insight into patterns of resistance[2], and evaluate strategies to mitigate toxicity[3]. Establishment of predictive biomarkers is critical to maximizing therapeutic benefits of immunotherapy[4]. Correlating biomarkers with clinical evidence will facilitate early identification of patients at risk of developing CRS and enhance efforts to safely deliver CAR T therapy[5].

The successful identification of biomarkers to achieve these goals will require assays that meet several criteria. The assay must be able to simultaneously measure a broad panel of analytes with a high degree of accuracy within a brief timeframe[1,6], and provide rapid and efficient evaluation of patient response[3].

The Ella immunoassay platform with Simple Plex™ multianalyte assays enable fast and accurate quantitation of analytes of interest and allow for the detailed analysis of an individual's response to T cell infusion. A large catalog of validated assays, and a small required sample size (25 µL) make this hands-free assay well-suited for characterizing dynamic molecular response. In order to assess the utility of Ella in this context, we analyzed samples from three CAR T infused donors (10 time points each collected over 13 days, including pre and post- treatment for each donor).

Methods

Serum samples from three individuals were collected over the course of 10 timepoints before, during, and following CAR T cell therapy. Investigators were blind to the medical information of these individuals. All samples were evaluated on the Ella multianalyte immunoassay platform for the following human biomarkers: Granzyme A, Granzyme B, IFN-γ, IL-6, IL-10, IL-15, IP-10, and TNF-α. Each sample was diluted according to specific kit instructions and mixed prior to assaying. Assay run time for each 4-analyte cartridge was approximately 1 hour. The concentrations of biomarkers in each sample were quantified by comparison to standard curves for each analyte, which were generated and pre-loaded onto each cartridge during manufacture. All data was obtained via triplicate results per biomarker per well.

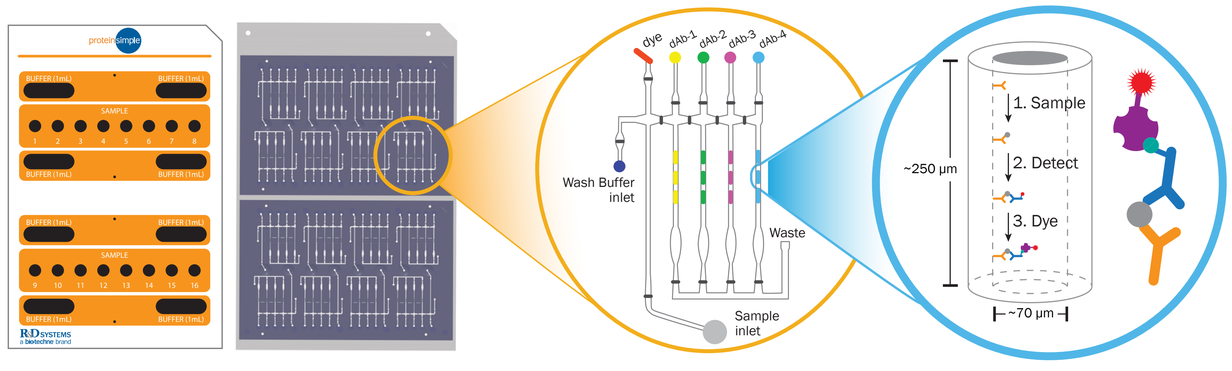

Figure 1. The Inner Workings of a Simple Plex Cartridge. Each sample well corresponds to a pneumatically-controlled microfluidic circuit, which contains 3 glass nanoreactors (GNRs) for each of 4 different analytes, along with all other necessary assay components. The inner diameter of each GNR contains analyte-specific capture antibody, while the remaining assay components are delivered pneumatically.

Results

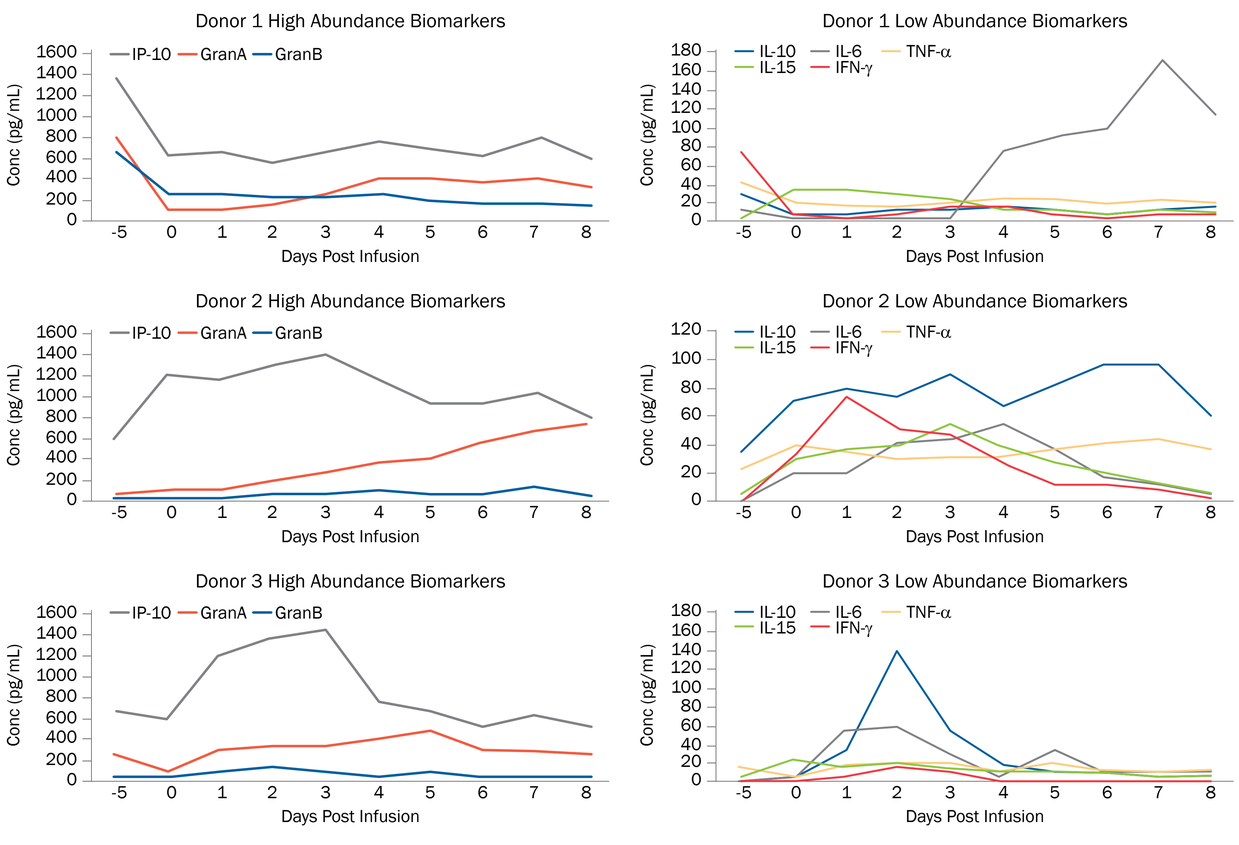

Figure 2. Each Donor Response Profile was Unique. Each donor presented a unique response profile. Elevated levels of many biomarkers were observed, with the degree of elevation and response time relative to T cell infusion varying between sample sets.

Range of Observed Sample Values in CAR T Donors vs Healthy Controls

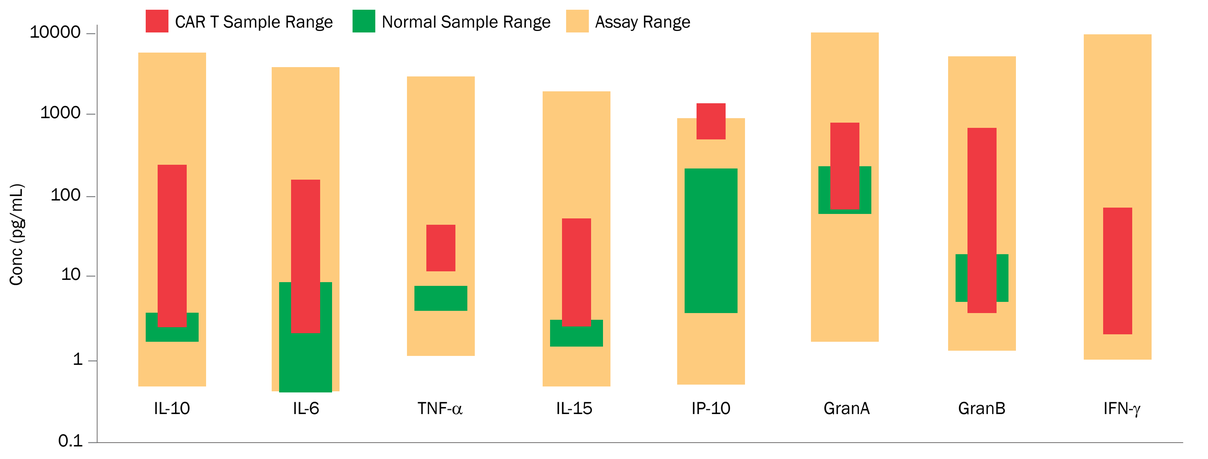

Figure 3. A Broad Range of Sample Values were Observed. The serum sample value range in the CAR T donor sample sets (n=3, 10 timepoints each) was broader than previously observed in healthy volunteers (n=10). The large dynamic range of the Simple Plex assays allowed for simultaneous quantitation of all analytes using a single dilution factor. Note that sample values shown here have been back calculated to account for dilution. IFN-γ levels in healthy controls were below the assay limits of quantitation.

Discussion

As research investigating the clinical utility of CAR T cell therapy continues, so too will the search for biomarkers which can be used to study subject response and assess potential risk. Our data, generated using Simple Plex immunoassay technology, shows biomarker response profiles varying considerably between donor sample sets (Figure 2). Donor 1 exhibited a uniquely elevated cytokine milieu prior to T cell infusion and showed little observable response until IL-6 increased drastically 4 days after infusion. By contrast, donors 2 and 3 exhibited increasing levels of IFN-γ, IL-6, IL-10, IL-15, and IP-10 within 24 hours of infusion. This heterogeneity of donor response, along with precipitous changes in cytokine abundance over short periods of time, highlight the importance of timely multianalyte analysis.

Collectively, the CAR T donor sample sets presented elevated levels for each biomarker compared to healthy controls. Sample value ranges were also broader in the CAR T donor sets compared to controls (Figure 3). The broad dynamic range of the Simple Plex assays allowed for quantitation of all biomarkers in all samples using a single dilution factor. Triplicate readings for each marker from every cartridge well, along with the pre-loaded standard curve provided confidence in the quality and continuity of our data. Characterizing CAR T cell therapy biomarkers is a pursuit that demands robust, sensitive, and reproducible assays. In this context, the Ella instrument running Simple Plex Assays is an ideal platform.

References

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