In vitro immunogenicity assessment using fresh PBMCs isolated from healthy donor whole blood samples

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1 Introduction

Immune response is commonly induced by pathogens and can also be induced in response to certain drugs (e.g. therapeutic antibodies, recombinant proteins). The propensity of a drug candidate to induce an unwanted immune response (immunogenicity) can be a significant hurdle during drug development and needs to be assessed early in drug development to minimise program risk. In this study, human primary PBMCs isolated from healthy donor whole blood were used as predictive models for immunogenicity assessment.

2 Methods

Fresh human venous blood samples from healthy donors were delivered in heparinized vials. PBMCs were isolated from whole blood within 3 to 4 hours of collection and were resuspended in RPMI culture media. Cells were plated in to a 96 well plate and incubated overnight for resting in a humidified 37°C, 5% CO_2 incubator. Following resting period, cells were treated with test agents for 24 hours in the incubator. 24 hours post treatment, cell supernatant was collected and analysed for cytokine levels using Luminex based Milliplex assay kit from MilliporeSigma.

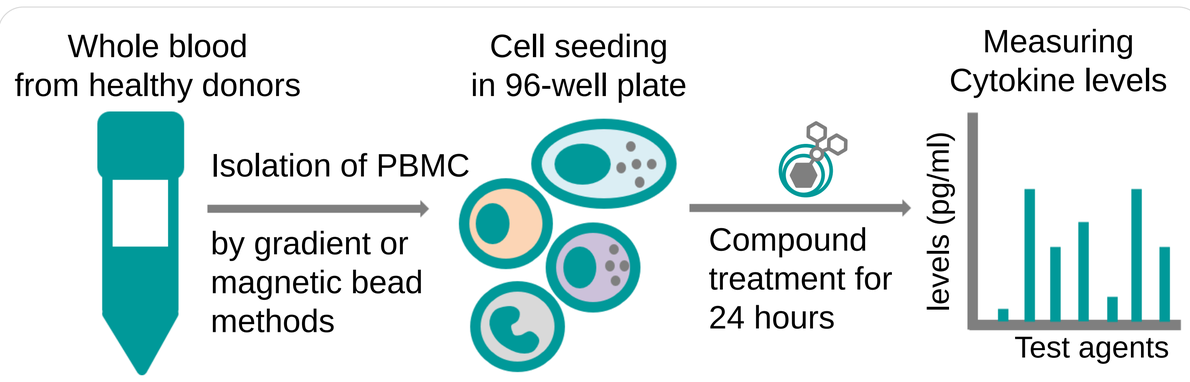


Figure-1: Isolation of PBMCs and immunogenicity assay method

3 Results

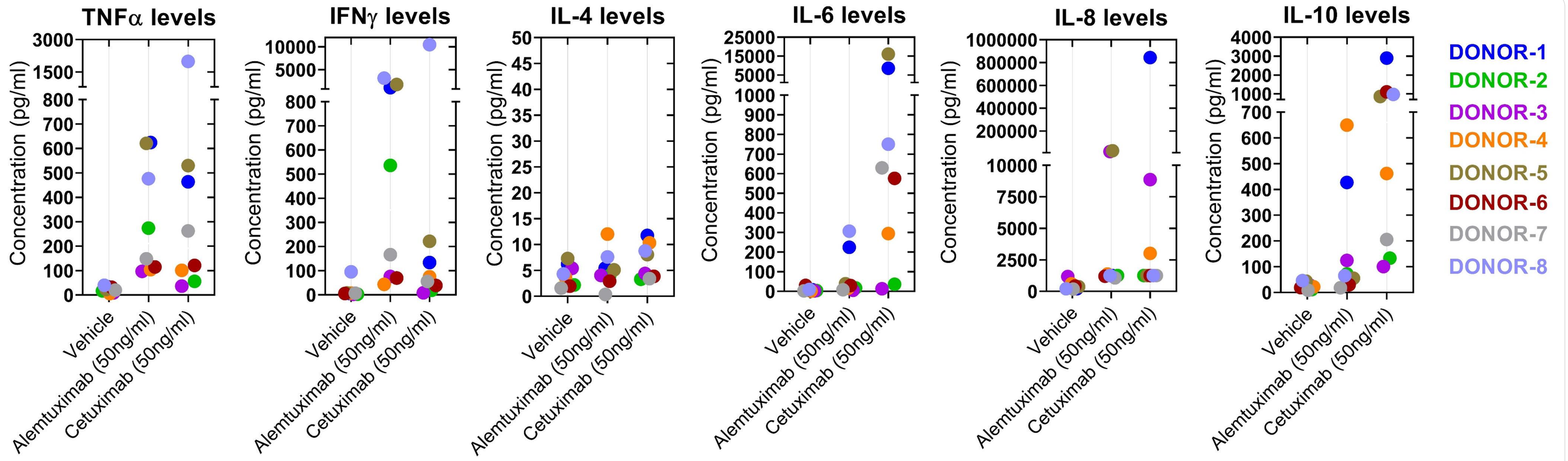


Figure-2: Concentration of each Cytokine analyte (pg/mL) in multiple donor samples. Data was plotted as y-axis (cytokine levels in pg/ml) versus x-axis (test condition). Data points within each donor were represented with one colour code. Each point represents average data from duplicate wells.

Cytokine induction fold increase across multiple donors

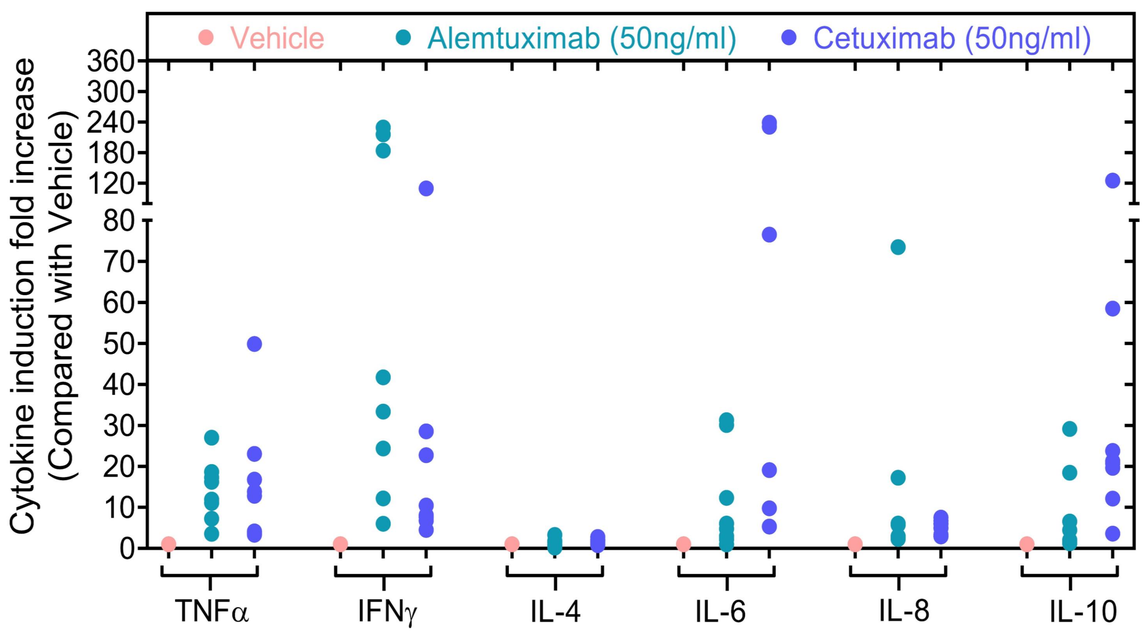
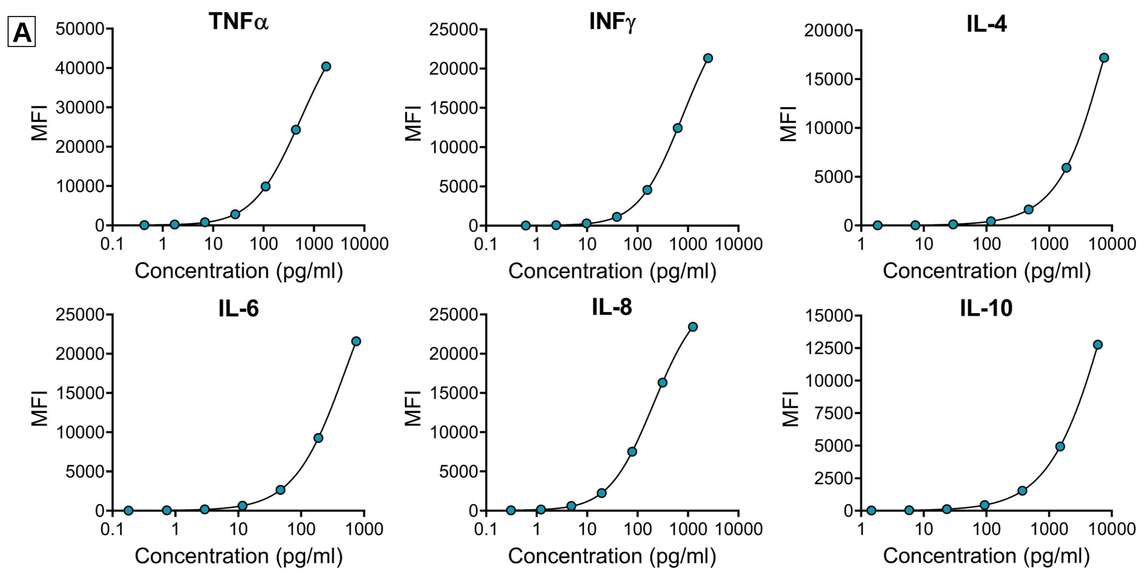


Figure-3: Cytokine induction fold increase against eight donors. Cytokine levels (pg/ml) data shown in Figures-2 was re-analysed to measure Cytokine induction fold increase in the presence of agents over vehicle control. Data was plotted as y-axis (Cytokine induction fold increase over Vehicle) versus x-axis (test condition within each cytokine). Each test condition performed against eight donors.

Quality controls: Standard curves and % Recoveries



B

% Recoveries of quality control replicates

	TNFα	IFNγ	IL-4	IL-6	IL-8	IL-10
Quality Control 1 (Low)	96	88	87	90	86	87
Quality Control 1 (Low)	98	91	87	90	86	87
Quality Control 2 (High)	107	93	95	99	98	104
Quality Control 2 (High)	104	91	92	96	95	103

Figure-4: **[A] Cytokine standard curves:** Data was plotted as y-axis (Mean Fluorescence Intensity=MFI) versus x-axis (cytokine concentration in pg/ml); **[B] % Recoveries** of quality control replicate samples.

4 Conclusions

The response exhibited by Alemtuzumab and Cetuximab in this assay agrees with clinical immunogenicity findings in the literature. *In vitro* immunogenicity assays developed at BioMedha are robust tools for implementing and assessing immunogenicity risk at an early stage. Understanding and managing immunogenicity at the earliest possible stage is important to improve safety and efficacy of therapeutic molecules in drug development.