Inclusion of exploratory PD biomarkers in first-in-human clinical trials adds value by providing information to confirm the mode of action of a candidate therapeutic

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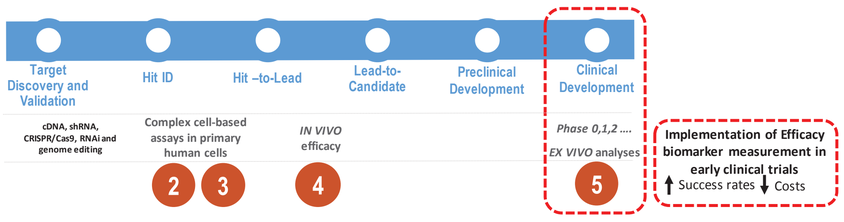
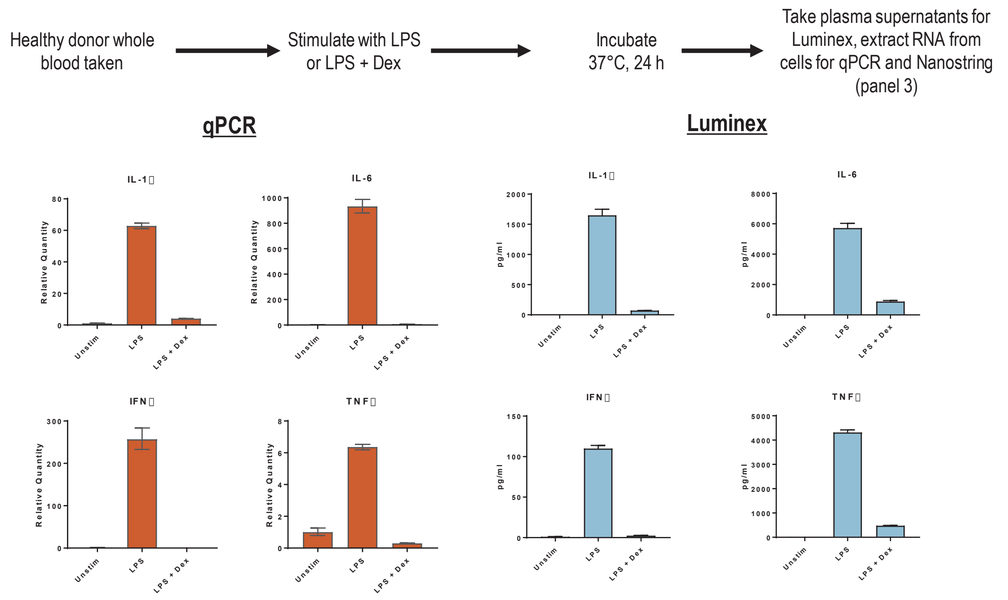
1 Introduction

Traditionally, in First-in-Human clinical trials the primary/secondary endpoints are concerned with safety. By expanding the remit of these studies to include study of exploratory biomarkers it is possible to gain added value, as such biomarkers can indicate efficacy and confirm mode of action of the therapeutic. Building this information in from an early stage can assist in go/no-go decisions and can greatly increase the likelihood of success. We show here how we currently use this strategy to monitor biomarkers in a translation manner using LPS-induced immune responses as an example. *In vitro* assays using human whole blood and PBMCs are routinely used to screen test compounds and determine their influence at the gene and protein level, using qPCR, Nanostring and Luminex platforms. This can be followed by *in vivo* pharmacology PD models (small rodent LPS challenge) to further refine the compound selection and develop suitable readouts such as Multiplex cytokine analysis. When a candidate compound then progresses to First-In-Human clinical trials, we can support the inclusion of exploratory biomarkers at this stage using 'uplifted' Good Clinical Laboratory Practice (GCLP) level assay validation, to ensure reliable clinical data. At this stage, a more focussed panel of cytokines can be selected and measured either directly in plasma or secondary to an ex vivo stimulation phase. In addition the use of flow cytometry and ELISPOT supports investigations of T cell modulating therapies. Such a strategy provides early indications on whether a therapeutic is hitting its expected target in man.

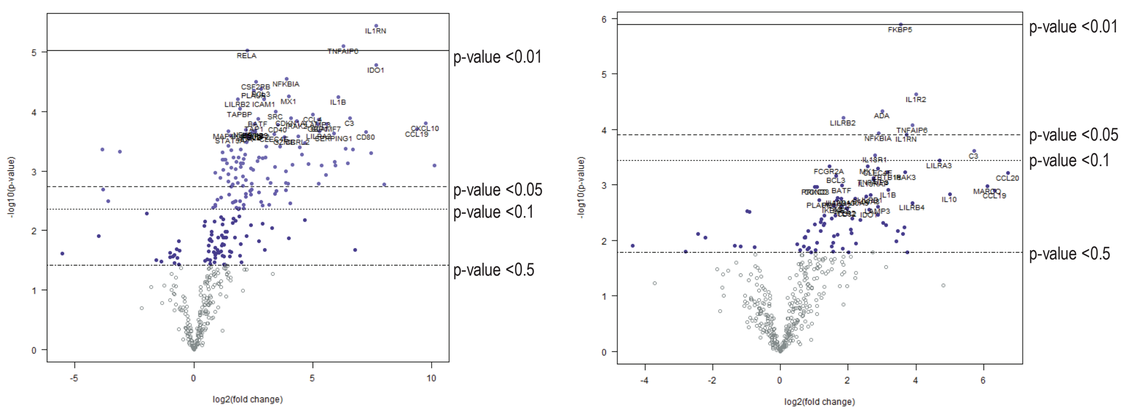


2 Pre-clinical *in vitro* analysis of inflammatory cytokine production to demonstrate target engagement



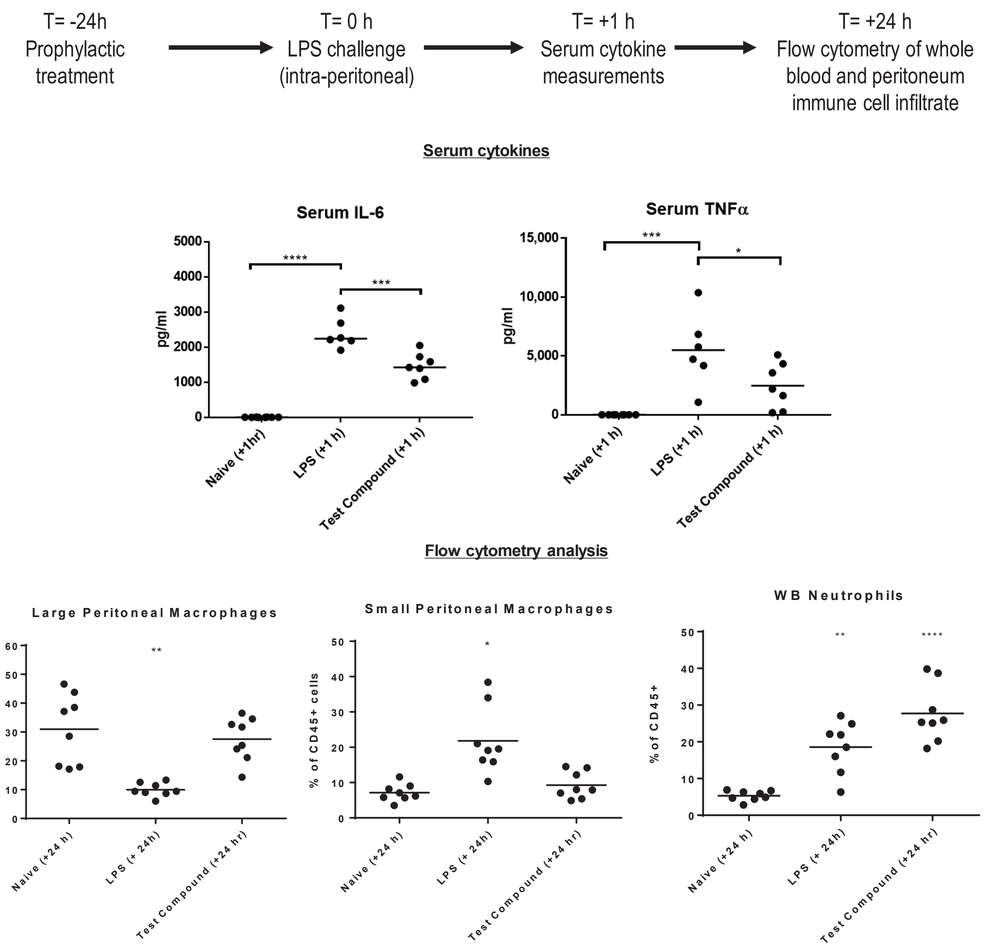
Novel therapeutics can be screened in *ex vivo* whole blood assays using healthy donor blood to demonstrate target engagement and to identify potential new biomarkers. Assay selection will be determined by predicted mechanism of action of the therapeutic. Inflammatory cytokine production can be screened at both the mRNA and protein level, by qPCR and Luminex, respectively. As illustrated here, for the cytokines shown, the two techniques show good concordance.

3 Nanostring analysis of gene expression profile in stimulated whole blood identifies potential clinical biomarkers



- Gene expression profiling (up to 800 targets) performed using Nanostring technology can be informative for biomarker selection, for validation of a pre-selected biomarker, or if wider pathway analyses are required.
- Horizontal lines indicate adjusted p value cutoffs. The further up the graph, the more statistically significant the change. Downregulated genes fall to the left, upregulated a genes are plotted further to the right.
- As expected, LPS induces upregulation of many genes (left), inhibition of LPS driven inflammation with dexamethasone reduces the significance and magnitude of differential gene expression (right).

4 *In vivo* pharmacology to demonstrate efficacy – LPS-induced peritonitis (murine)



- Selected test compounds are progressed to efficacy testing in small rodent models.
- Analytical readouts include serum cytokine analysis and flow cytometry of peritoneal washes and whole blood.
- LPS challenge in mice is characterised by a decrease in percentage of resident large peritoneal macrophages, and an influx of small peritoneal macrophages.
- The test compound demonstrates efficacy by reversing this trend in the peritoneum.
- Neutrophils can also be enumerated in whole blood.

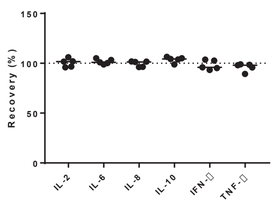
5 Exploratory end points supporting first-in-human clinical trials assists in critical decision making and provides information on candidate efficacy

Fit-for-purpose Validation
- Intra-assay precision
- Inter-assay precision
- Assay range (LLOQ, ULOQ)
- Recovery after spiking
- Recovery after long-term storage (mimic study logistics)
- Inter-operator variation

Inter-assay precision

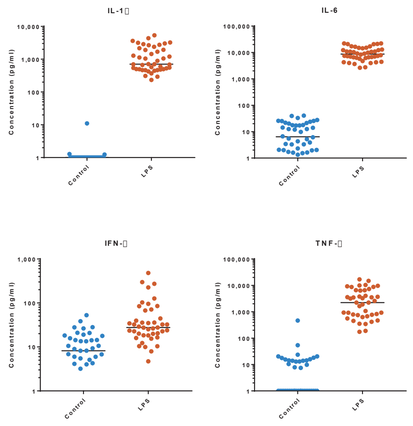
		Donor	TNF-α (pg/ml) Run 1	Run 2	Run 3	Average
24 h	Unstimulated	1	7.92	<LLOQ	<LLOQ	**7.92**
		2	<LLOQ	<LLOQ	<LLOQ	**N/A**
		3	9.37	8.24	7.91	**8.51**
	LPS, 10 ng/ml	1	5758.31	6115.79	5292.80	**5722.30**
		2	1174.94	973.56	870.47	**1006.32**
		3	1482.64	1454.15	1455.39	**1464.06**
	Activation QC (LPS)		3171.55	2833.74	2729.75	**2911.68**
	Plate QC (LPS)		3213.07	2776.71	2869.49	**2953.09**

Recovery from freeze-thaw (1 month)



Clinical Sample Analysis

Inter-donor variability



- Once a candidate therapeutic progresses to early phase clinical trials, measurement of exploratory biomarkers is validated before patient recruitment begins. Bespoke "fit-for-purpose" validation is performed for each trial, routinely to a higher level than is required for pre-clinical studies. During this validation phase, the trial logistics are mimicked to establish assay reproducibility.
- For example, depending on the trial logistics, samples may be collected in batches, stored and then analysed when a large enough pool of samples is available. Therefore, it is critical to know the biomarker recovery after storage. An understanding of inter-assay variability is also key.
- The graphs on the right show inter-donor variability in PBMC response to LPS stimulation in 45 healthy donors (activation on day of bleed, supernatants batched for measurement).

6 Summary

- Data shows that implementing efficacy biomarkers in early clinical trials reduces costs and increases success rates in the development of novel therapeutics.
- The Analytical services team at Charles River Portishead specialises in developing biomarker assays that can be translated from pre-clinical through to early clinical studies.
- Inclusion of exploratory end points and biomarkers from the earliest possible stages of the drug development process can assist in go/no-go decisions, and provide crucial information on candidate efficacy.