TREATMENT-SPECIFIC IMMUNE PHENOTYPES IN PBMCS REVEALED BY nELISA HIGH-THROUGHPUT PROTEOMICS

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Background
High-throughput screening (HTS) programs are increasingly adopting high-content technologies that can better inform the selection of drug candidates early on in the pipelines. For cancer immunotherapy, proteomics tools to investigate interactions between cancer and immune cells compromise either content or cost, limiting access to phenotypic data. The affordable gold-standard in proteomics, the ELISA, has proven difficult to scale. At fault has been the cross-reactivity between ELISA reagents when multiplexing beyond a few dozen antibody pairs. Here, we describe the nELISA: a massively-parallelized high-throughput miniaturized ELISA with a content, cost and throughput amenable to HTS, and demonstrate its applicability to characterize immune phenotypes in co-culture systems.

Methods
To overcome the long-standing cross-reactivity issue, the nELISA uses DNA oligos to pre-assemble each pair of antibodies onto a spectrally barcoded microparticle set. The resulting reagents are fully-integrated nELISA sensors that can be read-out on commercial cytometers, enabling highly-multi-plexed and high-throughput analysis. Using this approach, we developed a comprehensive inflammatory panel containing 191 cytokines, chemokines, proteases, growth factors, and soluble receptors. Our results show that the nELISA can maintain single-plex specificity, sensitivity, and quantification as content is scaled to 191-plex. Furthermore, the nELISA performs at a throughput of 1536 samples/cytometer/day, yielding >300,000 data points in a single day, at a cost amenable to high-throughput screening.

Results
To demonstrate the nELISA’s utility in HTS, we ran the largest PBMC secretome screen to date, in which >7000 PBMC samples were treated with various inflammatory stimuli, and further perturbed with a selected library of 80 recombinant protein ‘perturbagens’. 191 secreted proteins were profiled in all samples, resulting in ~1.4M datapoints (figure 1A). The nELISA profiles were able to capture phenotypes associated with specific stimulation conditions, individual donors, and potent cytokine perturbagens. By compensating for stimulation and donor differences, we clustered perturbagens according to their effects on PBMC secretomes, identifying well-established cell responses such as Th1 or Th2. Novel phenotypic effects were also identified, such as distinct responses to the near identical CXCL12 alpha and beta isoforms (figure 1B). Interestingly, we observed important similarities between PBMC responses to the cytokine drugs IFN beta and IL-1 Receptor antagonist, supporting the use of anakinra as a replacement for IFN beta in certain indications.

Conclusions
The nELISA captures broad secretome ranges and subtle differences in immune phenotypes, revealing critical insights in cell-based screens. Thus, the nELISA is a powerful new tool for cancer immunotherapy assays, including phenotypic screening, target identification/deconvolution, and discovery of markers of target engagement.

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High-throughput screen of PBMC responses demonstrates the use of the nELISA for drug discovery. (A) Screen design: PBMCs isolated from six donors were treated with inflammatory stimuli at indicated concentrations, and further perturbed with 80 recombinant cytokine “perturbagens”, generating a total of 7,392 samples; after 24 hours, concentrations of 191 secreted proteins were measured in the supernatant of each sample using the nELISA. (B) UMAP dimensionality reduction of the entire nELISA dataset; datapoints are colored (from left to right by stimulation condition, by donor, by stimulation concentration, or by individual cytokine perturbagens with strong effects, as indicated.