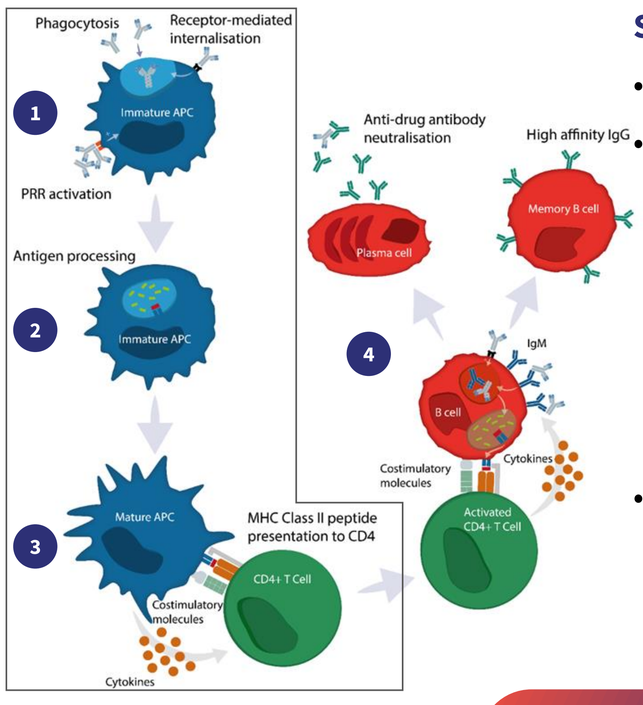
Next-Generation EpiScreen® Immunogenicity Assay

Mercedes Perez-Olivares*, Beverley Campbell, Edward Cloake, Erika Kovacs & Campbell Bunce
Abzena, Babraham Research Campus, Cambridge, UK
*** Email: mercedes.perez@abzena.com**

Introduction

Unwanted immunogenicity can affect the success of many protein therapeutics; thus, appropriate assessment is crucial before progressing a drug into the clinic. Using an assay with suitable sensitivity to evaluate this is key, and performed early in development can greatly benefit the developability of the therapeutic and its future chances of success. Here, we present an alternative method to traditional radioactive labelled markers using flow cytometry that does not compromise on the sensitivity. Additionally, the use of flow cytometry allows further characterization of the responding immune cells by multiplexing the readout with cell activation markers to increase confidence in immunogenicity risk assessment.

T cell epitopes and immunogenicity

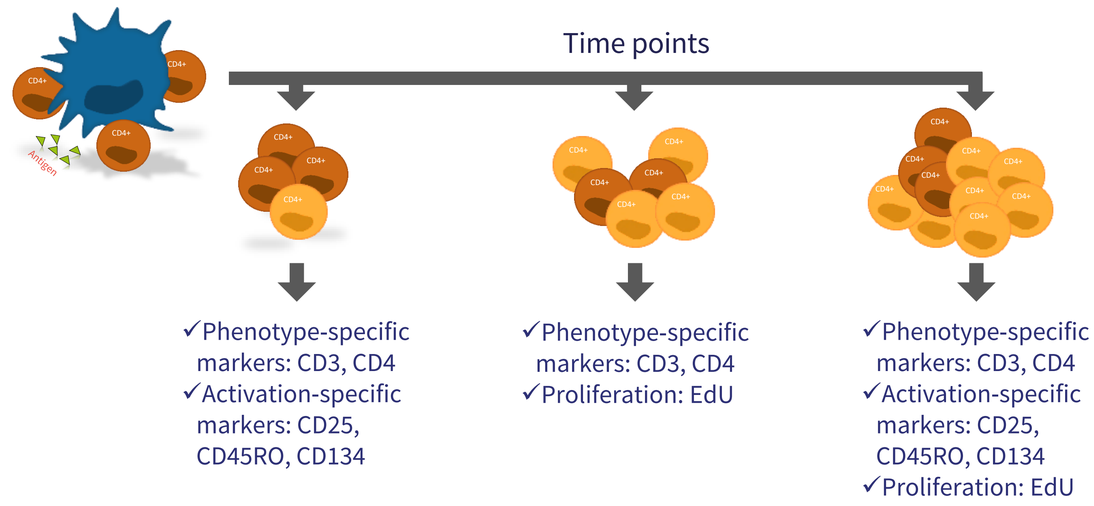


Simplified process overview

- Uptake of drug by APCs. [1]
- Linear peptides derived from drug during antigen-processing form complexes with MHC class II and activate T cells. [2] [3]
  - Note: peptide drugs (linear) could bypass processing by APCs and form complexes directly with MHC II molecules.
- T cell help ($CD4^+$ T cells) lead to high affinity, isotype switched anti-drug antibody (ADA) responses. [4]

Next-Generation EpiScreen® PBMC Time Course Assay

Purpose: To select a lead candidate from a number of variants and to confirm reduction in immunogenicity by assessing the whole molecule.



Abzena's immunogenicity assays interrogate the $CD4^+$ T cell response

Abzena's Next-Generation EpiScreen® PBMC Time Course Assay

Sensitive – Similar to [^{3}H]-thymidine method, can detect low frequency responses.

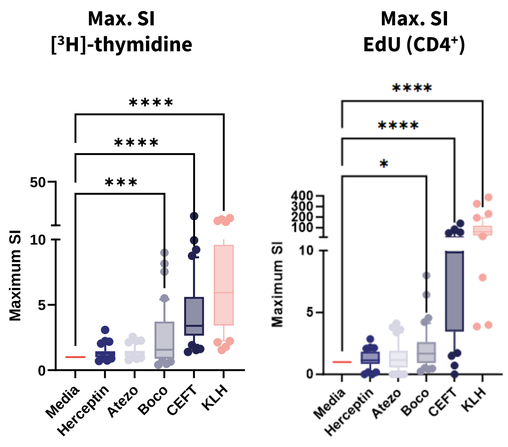


Figure 1. Box and whisker plot showing maximum proliferation SI obtained on PBMC over the time course in response to the samples. One-way ANOVA, followed by Friedman's post-test was used for statistical analysis. Atezo = Atezolizumab, Boco = Bococizumab. *p <0.05, ***p <0.001, **** p <0.0001. n = 40.

Specific – Specifically monitors $CD4^+$ T cell proliferation.

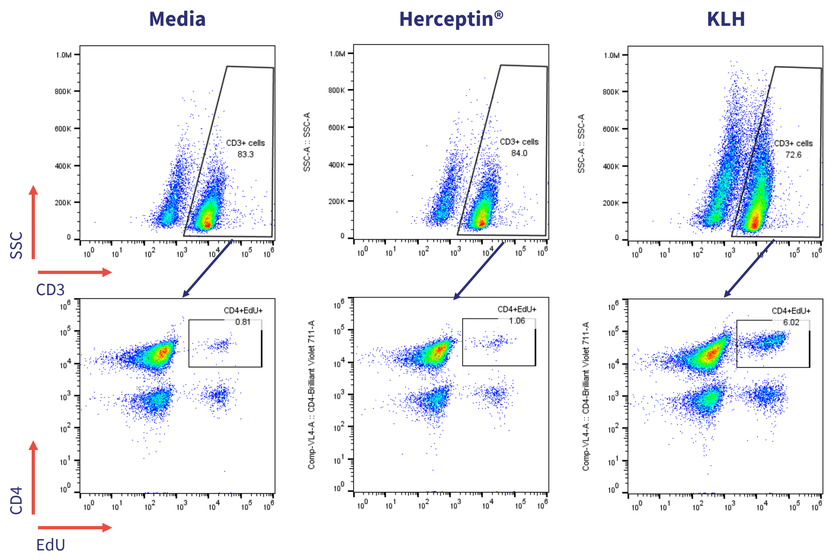


Figure 2. Flow cytometry gating strategy to determine EdU^+ cells in response to Media, Herceptin® (low immunogenicity control) and KLH (high immunogenicity control).

MoA-reflective – Provides information on $CD8^+$ T cell behavior.

Ability to monitor $CD8^+$ activation offers broader scope for immunogenicity analysis, particularly for gene therapy where vectors can enter an indirect antigen processing pathway.

Customisable – Option to modify and extend cell-surface markers to monitor other cell populations or activation mechanisms of your choice.

Data-rich – Assesses activation markers in addition to cell proliferation, provides more information on the triggered response and confirms activation of $CD4^+$ T cell populations.

CD134

- T cell co-stimulatory receptor from the TNF superfamily.
- Rarely present on unstimulated (naïve) T cells in human blood.
- Transient expression on both CD4+ and CD8+ T cells upon antigen stimulation.

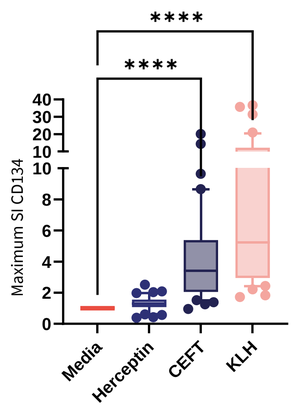


Figure 3. Box and whisker plot showing maximum proliferation SI obtained on $CD3^+$ $CD4^+$ T cells over the time course in response to the samples. One-way ANOVA, followed by Friedman's post-test was used for statistical analysis. **** p <0.0001. n =40.

CD45RO and CD25

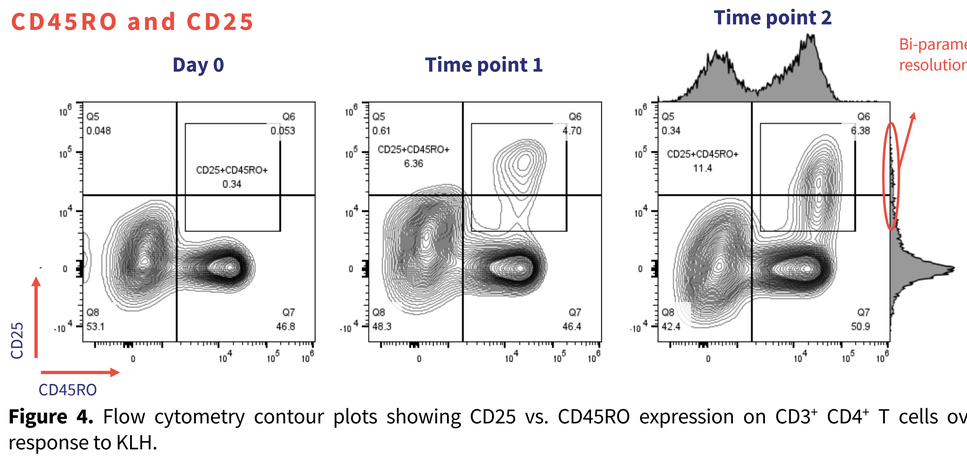
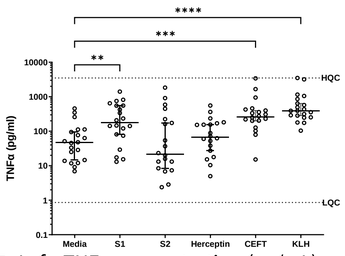
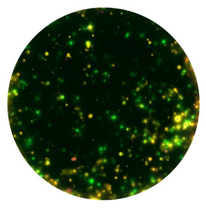


Figure 4. Flow cytometry contour plots showing CD25 vs. CD45RO expression on $CD3^+$ $CD4^+$ T cells over time in response to KLH.

Complementary assays

Cytokine Analysis by Luminex xMAP or FluoroSpot – Standardized or fit for purpose cytokine analysis to assess PBMC responses to a given drug candidate can augment interpretation of immunogenicity risk.





Example FluoroSpot well
Green = IFNγ,
Yellow = Granzyme B,
Red = IL-2

Figure 5. Left, TNFα concentration (pg/mL) on PBMC supernatants in response to the samples. **p <0.01, ***p <0.001, **** p <0.0001. Right, Representative FluoroSpot image of stimulated PBMC.

Summary

Understanding the potential immunogenicity of a drug candidate is a key step in progressing through to clinical trials.

Abzena is a market leader in immunogenicity assessment and has developed a comprehensive suite of assays to evaluate potential risks of clinical immunogenicity.

Assessing immunogenicity using our flow cytometry platform can allow activation markers to be observed alongside cell proliferation without compromising on assay sensitivity.

Introducing a data rich platform supports interpretation of immunogenicity risk including for advanced therapies (e.g., gene therapy) and improves selection of the best drug candidate for clinical evaluation.