Fit-for-purpose validation of an *in vitro* immunogenicity risk assessment assay

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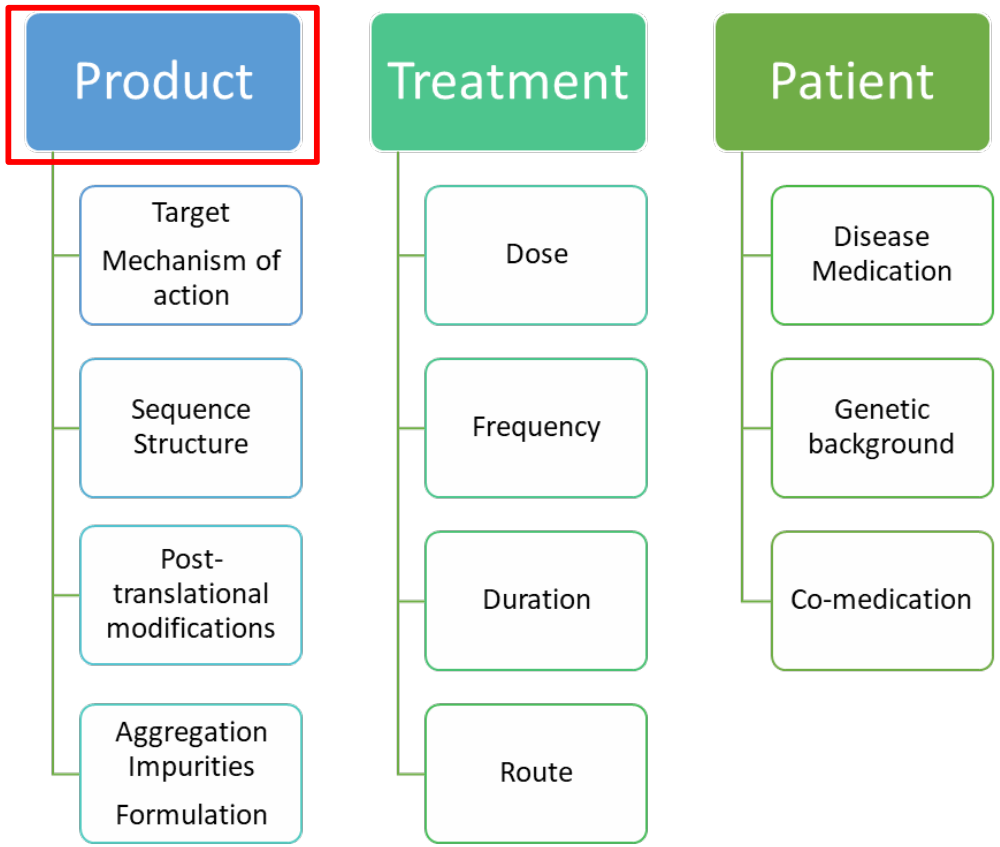
BioMedicine Design

Mitigation of biotherapeutic immunogenicity is needed to ensure benefit to patients, maintain commercial value and reduce attrition

Multiple factors influence Immunogenicity



Mitigation by design

In silico and *in vitro* assays guide molecular design by assessing risks at key steps in the immune cascade, which can lead to ADA development

B cell proliferation/differentiation assay

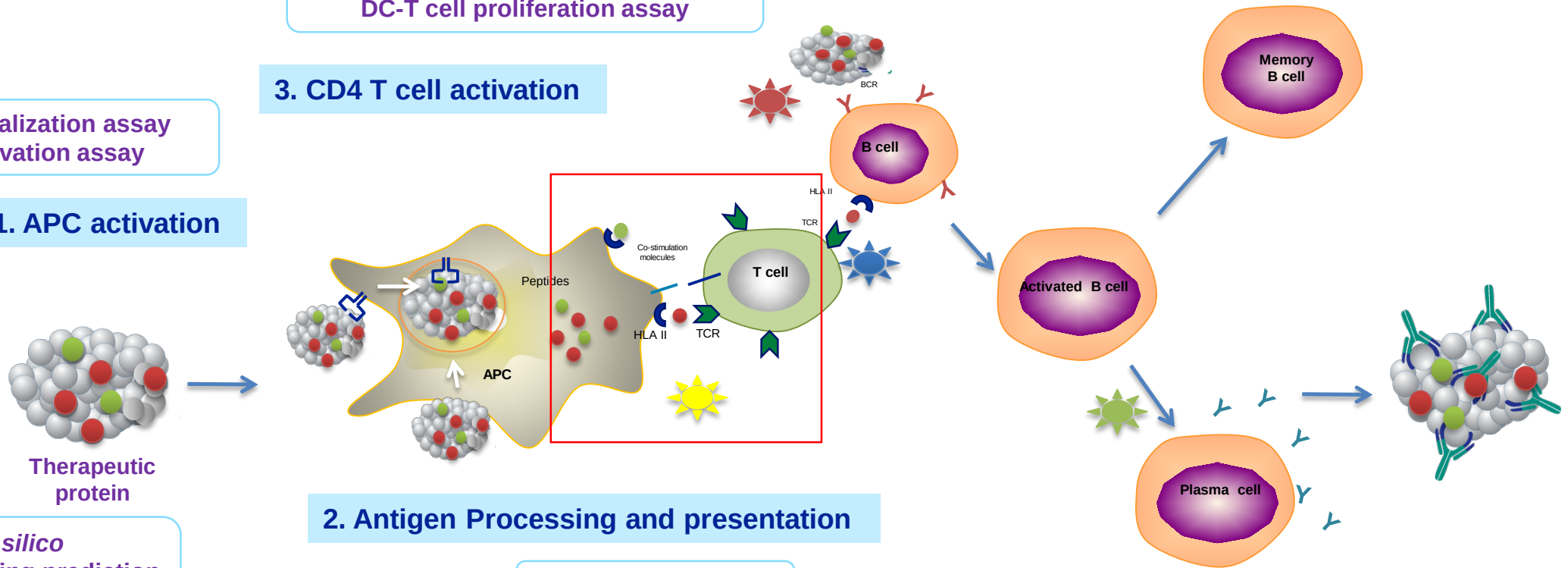
4. B cell activation & differentiation

PBMC T cell proliferation assay
DC-T cell proliferation assay

3. CD4 T cell activation

DC internalization assay
DC activation assay

1. APC activation



Therapeutic protein

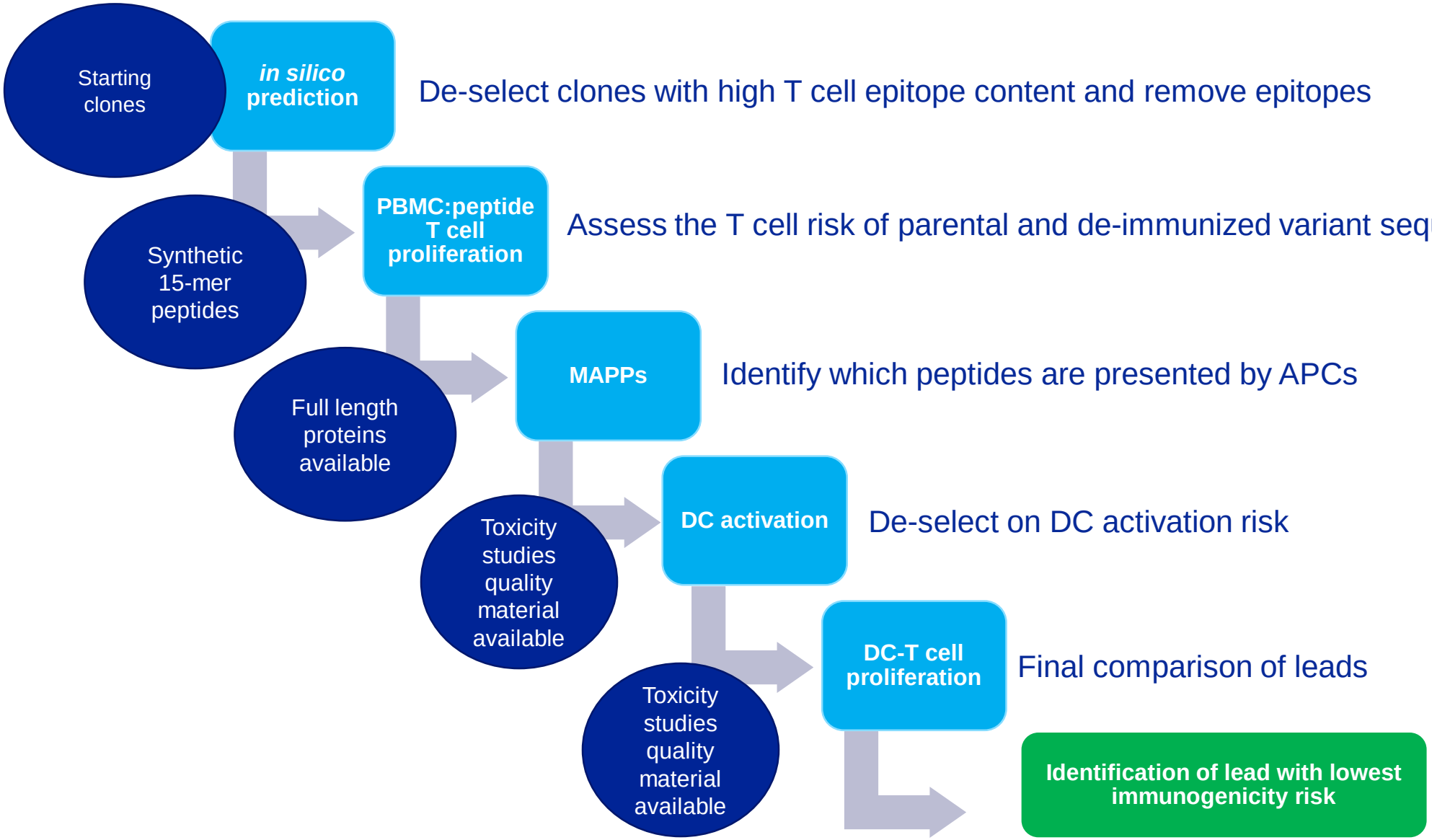
2. Antigen Processing and presentation

in silico
- HLA binding prediction
- Peptide presentation

MAPPs assay

ADA secretion

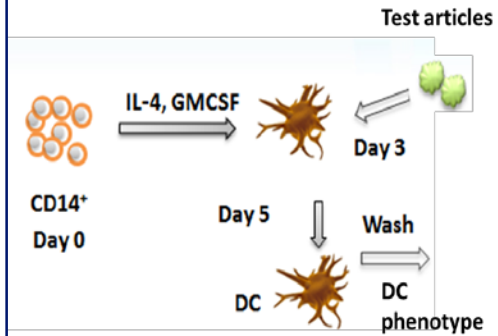
An assay-suite can be applied to guide molecular design and lead selection



DC activation assay principle & output: Pre-FFP validation

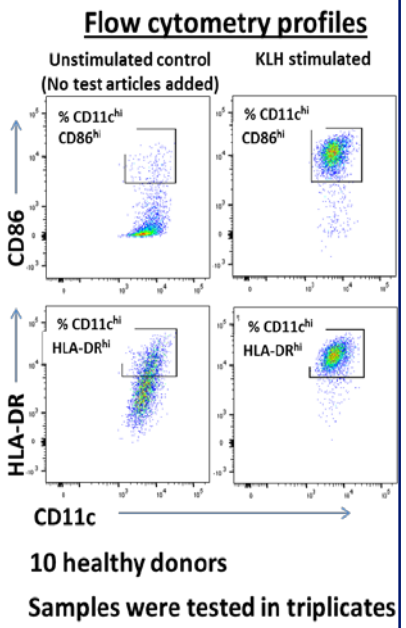
PRINCIPLE

Monocyte-derived DCs are grown from healthy donor cryopreserved PBMCs, incubated with test article and increase of activation markers is measured by flowcytometry



Flow cytometry Readouts:
Identify DC: CD11c

3 Flow cytometry readouts for DC activation:
% $CD86^{hi}$ $CD11c^{hi}$ DCs
% $HLA\text{-}DR^{hi}$ $CD11c^{hi}$ DCs
% $CD40^{hi}$ $CD11c^{hi}$ DCs



10 healthy donors

Samples were tested in triplicates

READOUTS

Stimulation Index (SI) = Test article treatment response / unstimulated control

$$SI = \frac{TA\ (\%)}{BC\ (\%)}$$

Positive response: SI ≥ 1.4

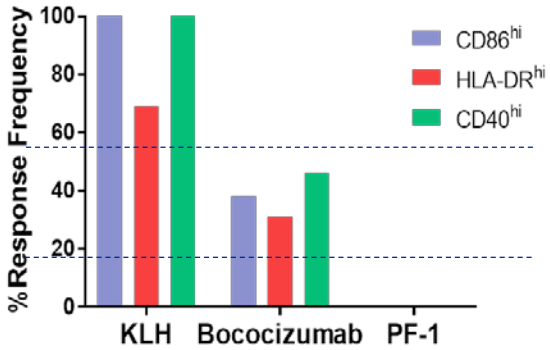
Donor response frequency = Number of donors with positive response for a test article / Total number of donors x100

$$\mathrm{RF}(\%) = 100\,\frac{\sum_{i=1}^{n} I(SI_i > 1.4)}{n}$$

OUTPUT

Risk ranking using donor response frequency; categories based on clinical relevance benchmarking

Response frequency ≤20%: Low risk
Response frequency >20-50%: Medium risk
Response frequency >50%: High risk

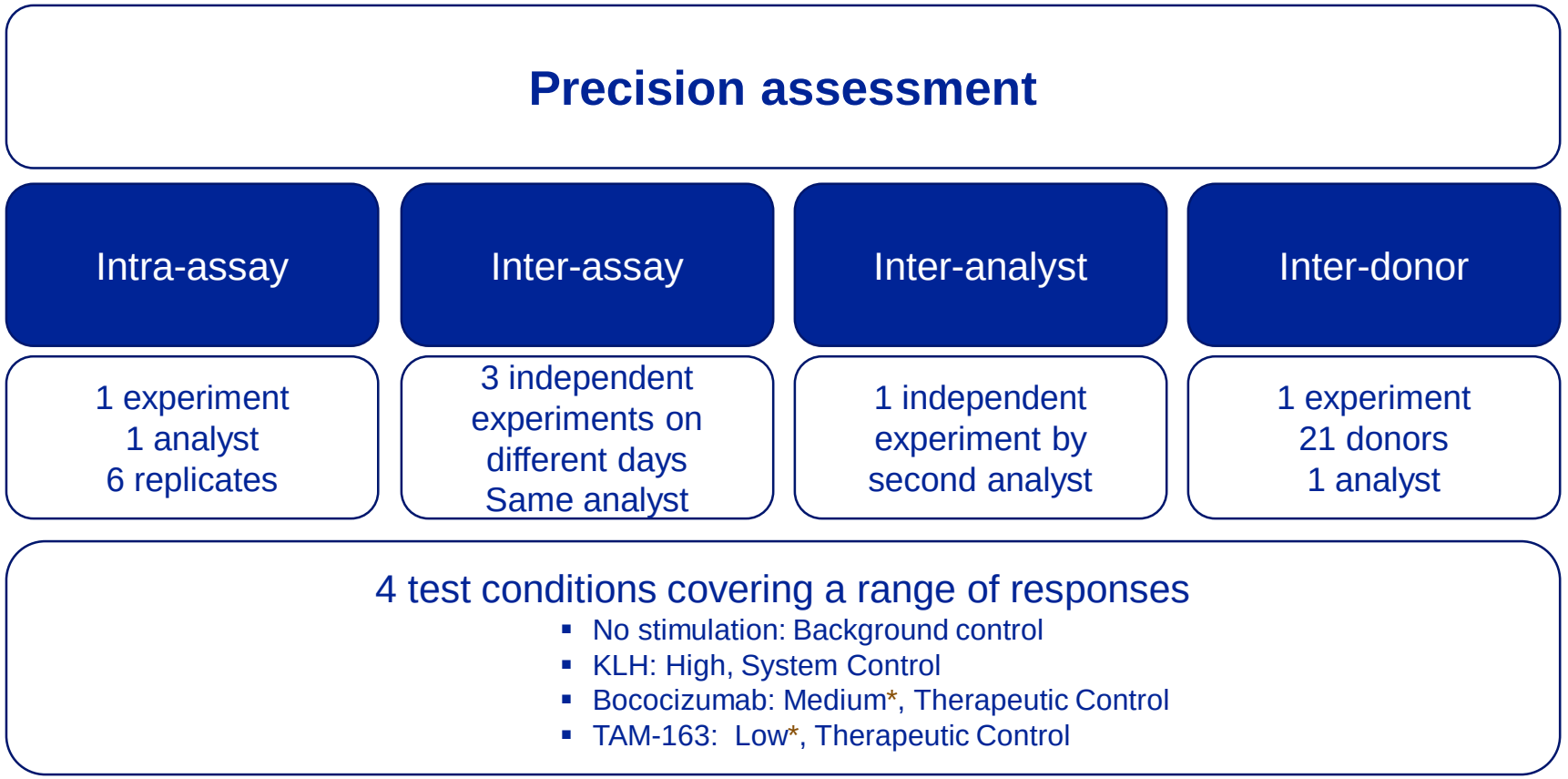


PF-1 low clinical immunogenicity therapeutic

The main objective of a fit-for-purpose validation is to characterize key parameters and assess performance of an assay

- Quantify assay precision (intra-assay; inter-assay; inter-analyst)
- Understand and control the contributing factors to assay signal variability
- Establish a positive response threshold
- Determine the minimum required donor cohort size
- Determine in-study donor acceptance criteria
- Establish a robust data reporting approach
- Track assay performance and quality over time

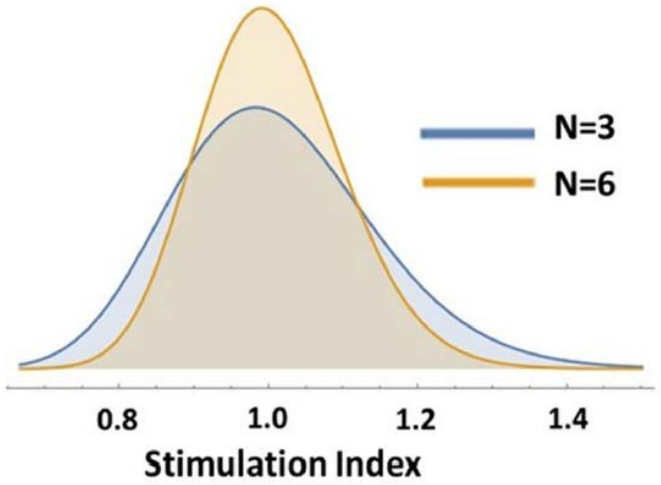
Key parameters characterization and assay performance can be derived from a controlled precision assessment study



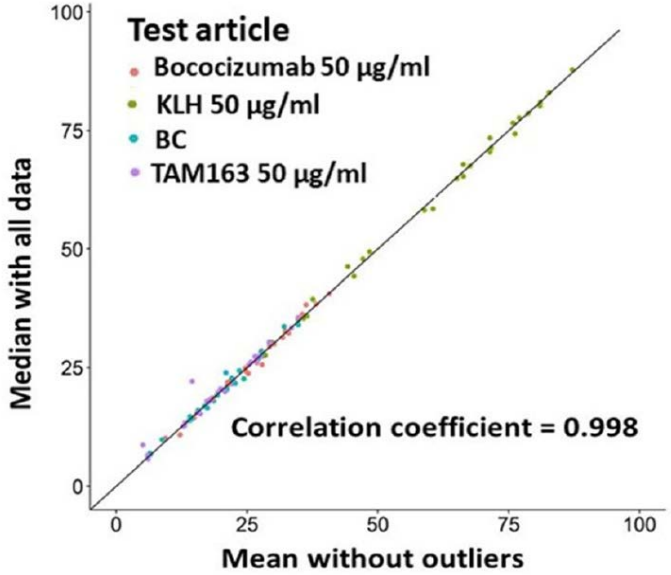
*Clinical ADA incidence previously characterized

Using median value of sample triplicates, the positive response cut-off is set at SI ≥ 1.4

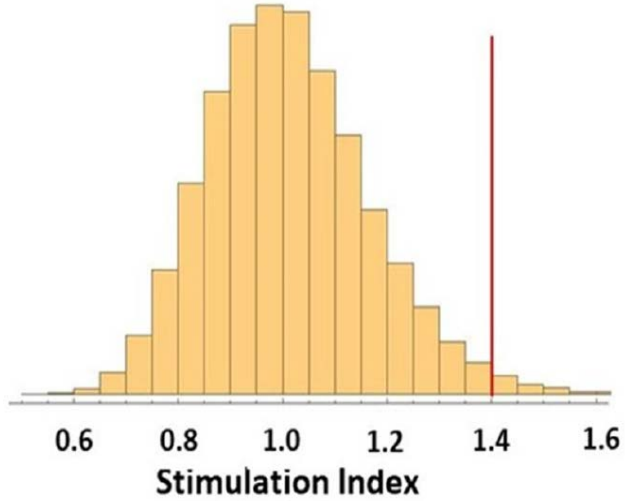
Sample triplication provides balance between precision and throughput



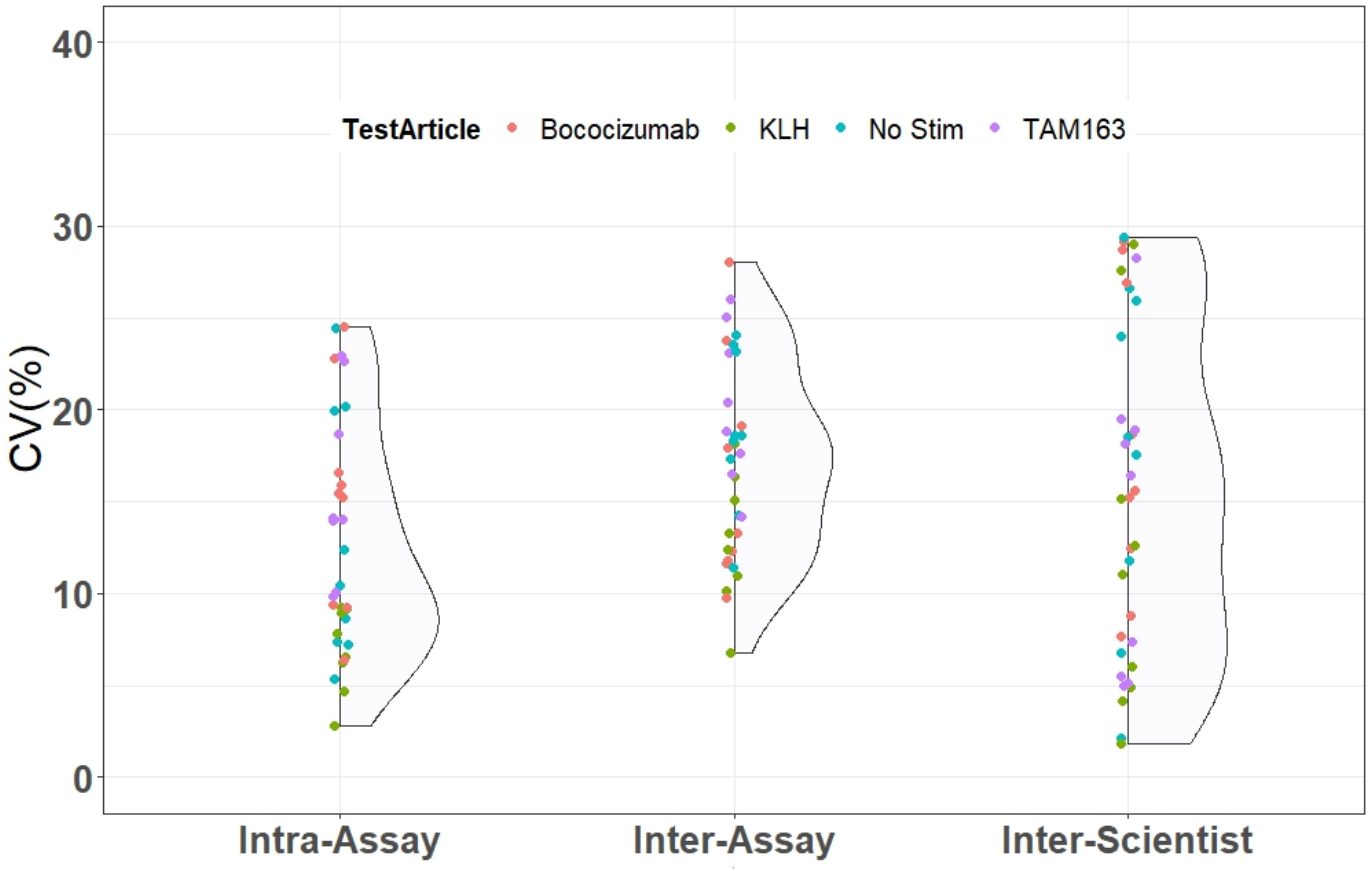
Use of median obviates the need for pre-determined acceptance criteria for identification of outliers



With SI ≥ 1.4 a test article gives a true response above background with 1% false positive rate

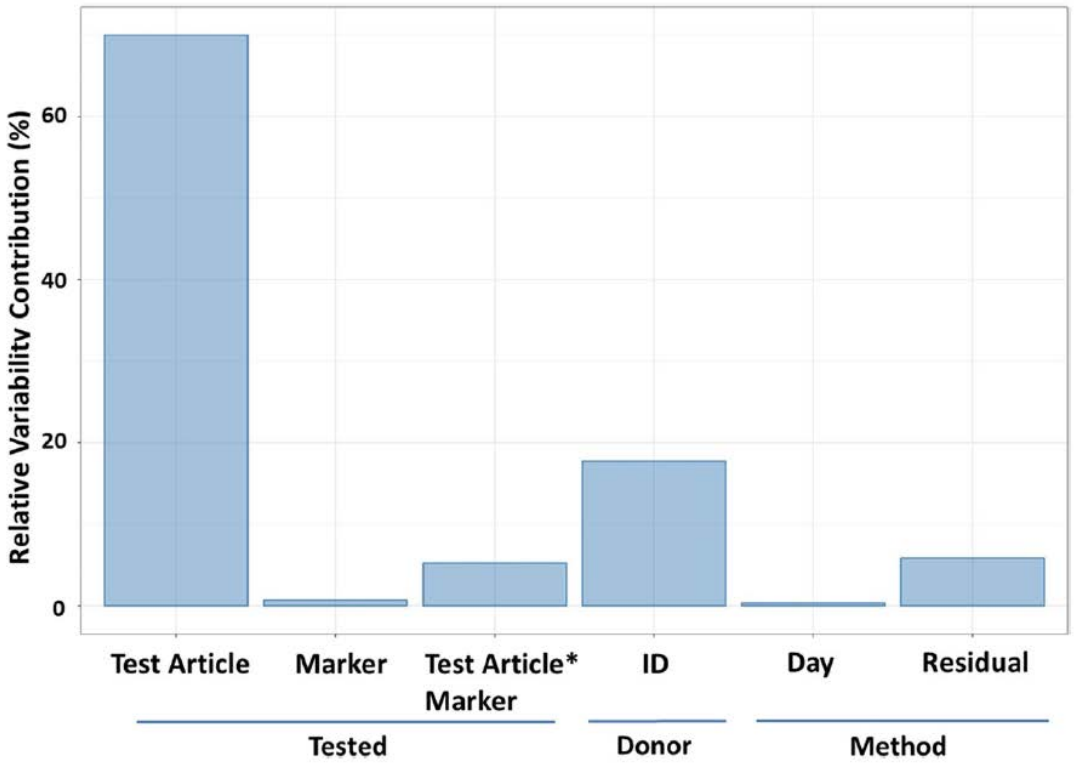


The overall SI precision meets the targeted %CV ≤ 30



DC Activation Assay SI Precision Data Distribution

Factors contributing to variability are suitable to enable test article differentiation using SI



Factors contributing to DC activation assay variability

Establishment of in-study donor acceptance criteria

- Data set: 21 donor panel from the validation + pre-validation experiments
- Performance factors considered:
  - Background DC activation
  - Response to System Control (KLH)

- Required for acceptance

  → Background activation ≤ 30%

  System Control SI ≥ 1.4 for all markers

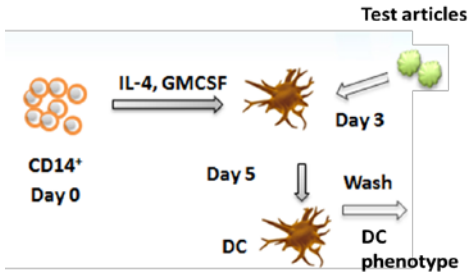
❖ Historical data demonstrate ~20% donors do not meet these acceptance criteria, hence each study is run with 15 donors to enable 10 reportable donors per study

Refined DC activation assay principle & output

PRINCIPLE

Monocyte-derived DCs are grown from healthy donor cryopreserved PBMCs, incubated with test article and increase of activation markers is measured by flowcytometry

50µg/ml



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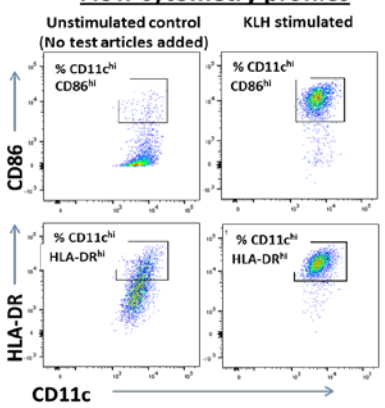
Flow cytometry profiles



Samples tested in triplicates

Median value

10 donor set

READOUT

Stimulation Index (SI) = Test article treatment response/ unstimulated control
Positive response: **SI ≥ 1.4**

Donor response frequency = Number of donors with positive response for a test article/ Total number of donors x100

DONOR ACCEPTANCE CRITERIA

Donor response to KLH:

- **Background activation ≤ 30%**
- **Positive response: SI ≥ 1.4 for all markers**

Test 15 donors to report a minimum of 10

OUTPUT

Risk ranking using donor response frequency; categories based on historical data evaluation for clinical relevance

Response frequency ≤20%: Low risk
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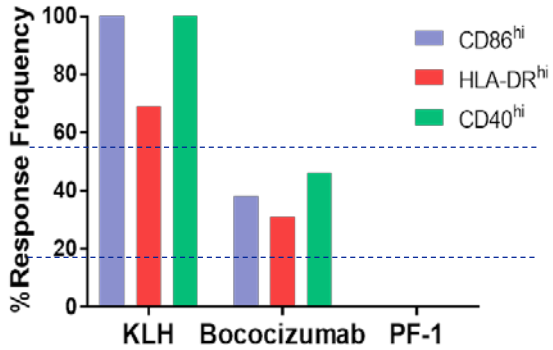
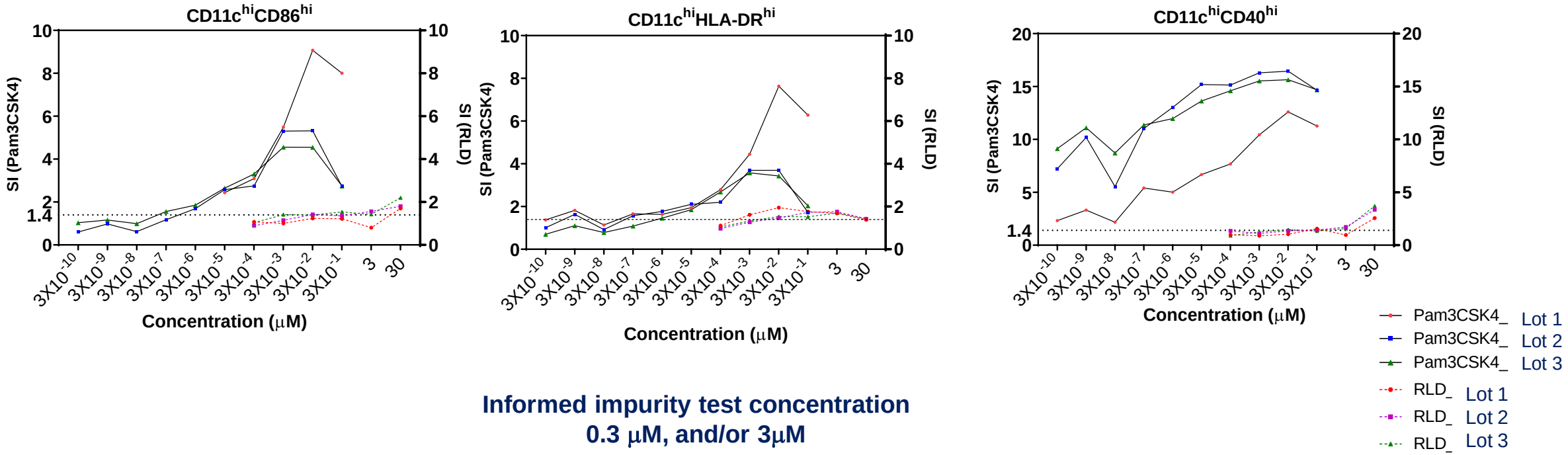


PF-1 low clinical immunogenicity therapeutic

The sensitivity of the assay to TLR activation could allow its use for immunogenicity risk of generic peptides impurities

Assay	Sensitivity	Control peptide
DC Activation	3pM	Pam3CSK4



Informed impurity test concentration
0.3 μM, and/or 3μM

Conclusion and outlook

The DC activation assay performance satisfies its intended use as an immunogenicity risk assessment screening tool for molecular design and lead selection of protein drugs

- Learnings from this FFP validation are being applied to other in vitro assays such as DC-T or PBMC:peptide T cell assays
- Further analysis of data continues to increase confidence in the tools
- The same FFP validation could be applied to the DC activation assay for intended use as an immunogenicity risk assessment tool for generic peptides
- A desirable next step is harmonization of methods across the field to allow comparability of results across laboratories. Working groups, such as the European Immunogenicity Platform's Non-Clinical Immunogenicity Risk Assessment working group (NCIRA) are discussing strategies and recommendations for such harmonization

Acknowledgment

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Tim Hickling

Fit-for-Purpose Validation and Establishment of Assay Acceptance and Reporting Criteria of Dendritic Cell Activation Assay Contributing to the Assessment of Immunogenicity Risk.
Wickramarachchi D, Steeno G, You Z, Shaik S, Lepsy C, Xue L
AAPS J. 2020 Aug 24;22(5):114

Thank you