HESI/AAPS Reference Panel for Preclinical Immunogenicity Risk Assessment

Laurent Malherbe

Executive Director, ADME

Eli Lilly and Company

On behalf of the

AAPS Immunogenicity Risk Assessment and Mitigation Working Group

HESI-Immunosafety Method Development Workgroup

EIP 14th Open Symposium, Lisbon, April 2023

Lilly

Outline

- Origins of Reference Panel
- HESI/AAPS Reference Panel Work Proposal
- Preliminary Results

AAPS Immunogenicity Risk Assessment & Mitigation Working Group

- Working group within AAPS Therapeutic Product Immunogenicity Community
- **Vision:**
  - *connect scientists working on preclinical immunogenicity risk assessment and mitigation*
  - *increase knowledge and understanding of the various risk assessment tools and evaluate their utility*
  - *provide a forum to discuss challenges facing the field*
  - *formulate and communicate industry opinions on regulatory challenges related to immunogenicity risk assessment"*
- Monthly teleconference (2nd Tuesday of the month)
- Membership:
  - 58 members (US / Europe, Industry/Academia / FDA)

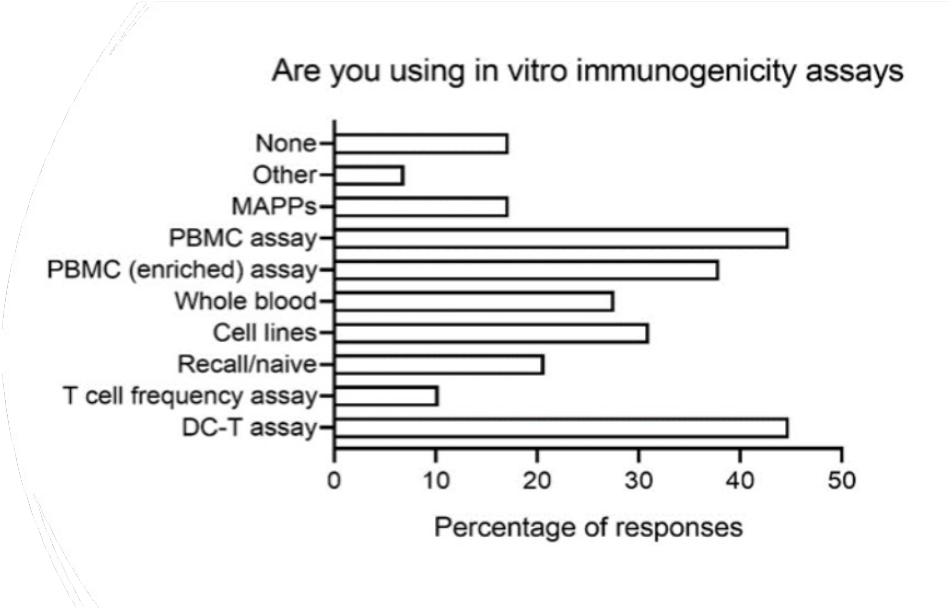
Chair: Sofie Pattijn
Former Chair: Laurent Malherbe
Vice Chair: Robin Walsh
Secretary: Daniel Leventhal

aaps® American Association of Pharmaceutical Scientists

Survey outcome on immunogenicity risk assessment tools for biotherapeutics: an insight into consensus on methods, application and utility in drug development

Jochem Gokemeijer,[1*#] Yi Wen,[2*] Vibha Jawa,[3*], Shibani Mitra-Kaushik[4], Shan Chung[5], Alan Goggins[6], Seema Kumar[7], Kasper Lamberth[8], Karen Liao[9], Jennie Lill[5], Qui Phung[5], Robin Walsh[2], Brian Roberts[10], Michael Swanson[11], Inderpal Singh[12], Sophie Tourdot[13], Mark A. Kroenke[14] Bonita Rup[15], Theresa J. Goletz[16], Swati Gupta[17], Laurent Malherbe[2], and Sofie Pattijn[18]

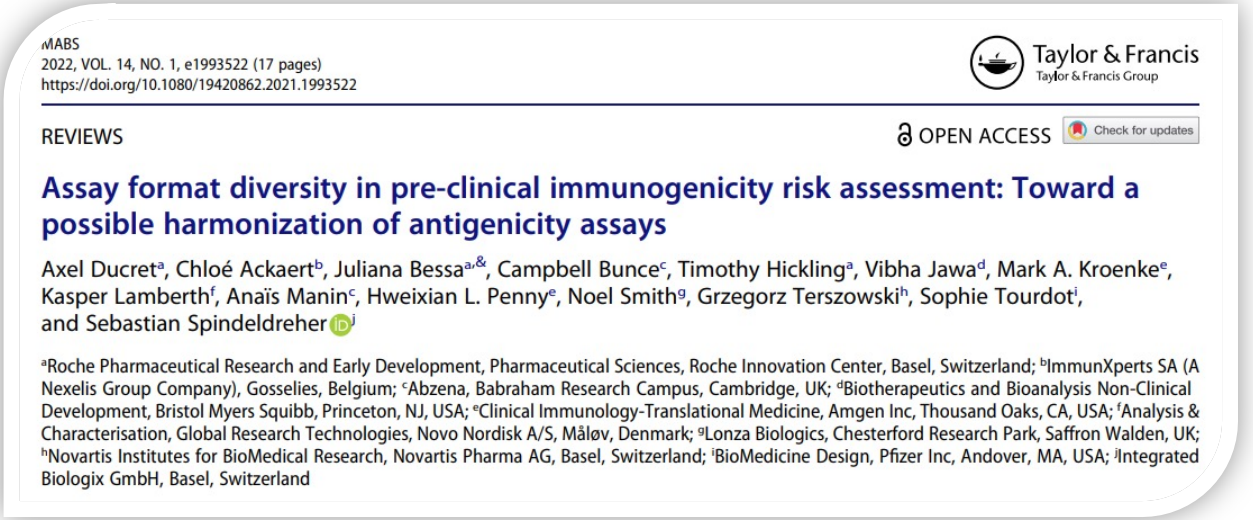
- 70% of the member companies used some type of predictive assay preclinically
- No alignment on the methods used or data interpretation and criteria used for reporting of predicted risk
- The biggest gaps to a broader implementation of immunogenicity screening were related to:
  - the translatability and predictive value of the preclinical risk assessment data in clinic
  - **lack of standardization and benchmarking**
  - high variability for these assays.



Slide courtesy of Yi Wen, Jochem Gokemeijer, Vibha Jawa and AAPS IRAM work group

Convergence between AAPS and EIP

"While the data generated by these assays and platform is becoming increasingly informative and valuable, the interpretation and translation into an overall immunogenicity risk of a given therapeutic protein remains challenging **due to lack of assay standardization** and harmonization".



MABS
2022, VOL. 14, NO. 1, e1993522 (17 pages)
https://doi.org/10.1080/19420862.2021.1993522

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Taylor & Francis Group

REVIEWS

OPEN ACCESS Check for updates

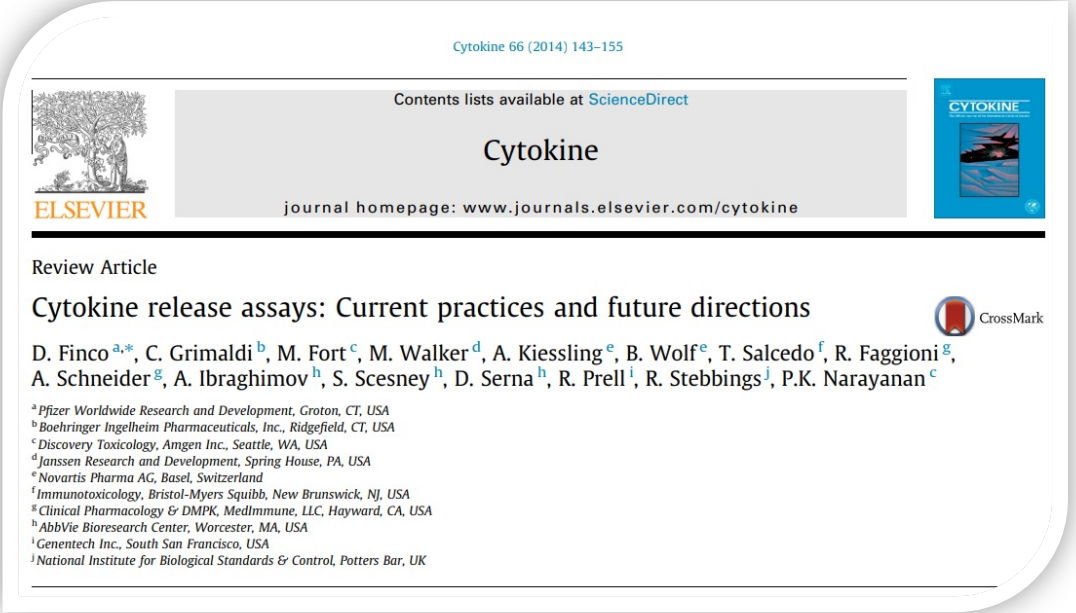
Assay format diversity in pre-clinical immunogenicity risk assessment: Toward a possible harmonization of antigenicity assays

Axel Ducret[a], Chloé Ackaert[b], Juliana Bessa[a,&], Campbell Bunce[c], Timothy Hickling[a], Vibha Jawa[d], Mark A. Kroenke[e], Kasper Lamberth[f], Anaïs Manin[c], Hweixian L. Penny[e], Noel Smith[g], Grzegorz Terszowski[h], Sophie Tourdot[i], and Sebastian Spindeldreher[j]

[a]Roche Pharmaceutical Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center, Basel, Switzerland; [b]ImmunXperts SA (A Nexelis Group Company), Gosselies, Belgium; [c]Abzena, Babraham Research Campus, Cambridge, UK; [d]Biotherapeutics and Bioanalysis Non-Clinical Development, Bristol Myers Squibb, Princeton, NJ, USA; [e]Clinical Immunology-Translational Medicine, Amgen Inc, Thousand Oaks, CA, USA; [f]Analysis & Characterisation, Global Research Technologies, Novo Nordisk A/S, Måløv, Denmark; [g]Lonza Biologics, Chesterford Research Park, Saffron Walden, UK; [h]Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland; [i]BioMedicine Design, Pfizer Inc, Andover, MA, USA; [j]Integrated Biologix GmbH, Basel, Switzerland

A sense of Déjà vu : Cytokine Release Assays Development (2014)

- Every pharmaceutical companies developed cytokine release assays to assess the risk of cytokine release syndrome after the cytokine storm experience with TGN 1412 in 2006.
- “It is clear from the HESI survey and follow-up, that there is no standard approach to strategies, assay formats and reporting and interpretation of data, and that pharmaceutical companies, CROs and academic institutions use a variety of approaches.”
- A ‘one-size-fits-all’ approach is not recommended because different biotherapeutics, based on varying mechanisms of actions, may require different approaches.
- However, alignment of technical procedures including positive and negative controls, for frequently used formats and data interpretation and its implementation, as well as regulatory expectations would allow a higher level of harmonization across the scientific community.



Cytokine 66 (2014) 143–155

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Review Article

Cytokine release assays: Current practices and future directions

D. Finco [a,*], C. Grimaldi [b], M. Fort [c], M. Walker [d], A. Kiessling [e], B. Wolf [e], T. Salcedo [f], R. Faggioni [g], A. Schneider [g], A. Ibraghimov [h], S. Scesney [h], D. Serna [h], R. Prell [i], R. Stebbings [j], P.K. Narayanan [c]

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[h] AbbVie Bioresearch Center, Worcester, MA, USA
[i] Genentech Inc., South San Francisco, USA
[j] National Institute for Biological Standards & Control, Potters Bar, UK

HESI-Immuno-safety Technical Committee

- **Health and Environmental Sciences Institute (HESI)** is a non-profit institution whose mission is to collaboratively identify and help to resolve global health and environmental challenges through the engagement of scientists from academia, government, industry, NGOs, and other strategic partners.
- **Immuno-safety Technical Committee**
  - Over 160 active individual active participants across industry, government and academic
  - **Mission:** To identify and address scientific issues related to immune safety and translation to human health risk assessment
  - **Key Objectives:**
    - Leverage technical and scientific expertise from academic, regulatory, and industry organizations to advance immuno-safety science
    - Contribute to the scientific decision-making processes relative to the development of guidelines and regulations for immune safety testing
    - Educate stakeholders in safety science and promote the understanding and appropriate use of immune safety data

HESI-ITC Method Development Workgroup

Method Development Workgroup Co-Leads: Courtni Newsome (BMS), Sandrine Vessillier (NIBSC)

Vision: To share, optimize methodologies used for immune safety testing between companies and research organization to assure appropriate preclinical data for public health safety

WHAT:

- Identify, inform on and assess emerging technologies used by research organizations to address safety liabilities of biologics , small molecules and cell therapy products
- Identify any issues/gaps in the models, assays and data interpretation
- Provide relevant controls to include in the assays

HOW:

- By prioritizing new projects in function of the scientific landscape priorities
- By sharing information on specific methods through survey, SharePoint, teleconferences
- By liaising with other WGs when overlapping interests
- By collaborating with education WG to organize webinar on specific methodology
- By publishing good practices guidelines for the scientific community

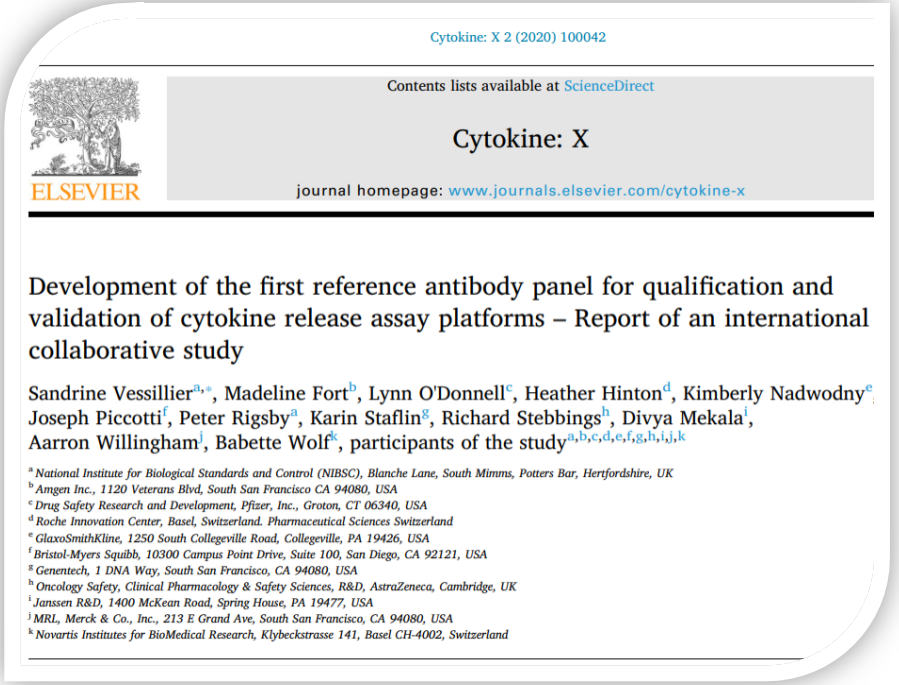
WHY:

- To assure a robust use of methods and correct data interpretation

HESI

Cytokine Release Assay Reference Panel

- “ One of the challenges for the development and comparison of CRA performance **is the lack of availability of standard positive and negative control mAbs for use in assay qualification**.
- To address this issue, the National Institute for Biological Standards and Control (NIBSC) developed a reference panel of lyophilised mAbs known to induce CRS in the clinic: human anti-CD52, mouse anti-CD3 and human superagonistic (SA) anti-CD28 mAb manufactured according to the respective published sequences of Campath-1H® (alemtuzumab, IgG1) , Orthoclone OKT-3® (muromonab, IgG2a) and TGN1412 (theralizumab, IgG4), as well as three isotype matched negative controls (human IgG1, mouse IgG2a and human IgG4, respectively).
- The relative capacity of these control mAbs to stimulate the release of IFN-γ, IL-2, TNF-α and IL-6 in vitro **was evaluated in eleven laboratories in an international collaborative study mediated through the HESI Immuno-safety Technical Committee Cytokine Release Assay Working Group**.”



Cytokine: X 2 (2020) 100042

Contents lists available at ScienceDirect

Cytokine: X

journal homepage: www.journals.elsevier.com/cytokine-x

Development of the first reference antibody panel for qualification and validation of cytokine release assay platforms – Report of an international collaborative study

Sandrine Vessillier[a,*], Madeline Fort[b], Lynn O'Donnell[c], Heather Hinton[d], Kimberly Nadwodny[e], Joseph Piccotti[f], Peter Rigsby[a], Karin Staflin[g], Richard Stebbings[h], Divya Mekala[i], Aarron Willingham[j], Babette Wolf[k], participants of the study[a,b,c,d,e,f,g,h,i,j,k]

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[b] Amgen Inc., 1120 Veterans Blvd, South San Francisco CA 94080, USA
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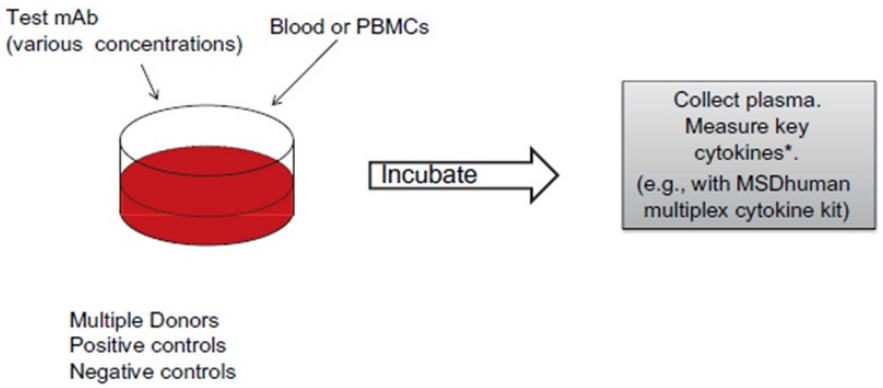
[d] Roche Innovation Center, Basel, Switzerland. Pharmaceutical Sciences Switzerland
[e] GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA
[f] Bristol-Myers Squibb, 10300 Campus Point Drive, Suite 100, San Diego, CA 92121, USA
[g] Genentech, 1 DNA Way, South San Francisco, CA 94080, USA
[h] Oncology Safety, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, Cambridge, UK
[i] Janssen R&D, 1400 McKean Road, Spring House, PA 19477, USA
[j] MRL, Merck & Co., Inc., 213 E Grand Ave, South San Francisco, CA 94080, USA
[k] Novartis Institutes for BioMedical Research, Klybeckstrasse 141, Basel CH-4002, Switzerland

Cytokine Release Assay Panel development
Assay diversity

Table 3
CRA performed by the participants.

Assay type	IncubationTime	Number of participants	Number of donors (all participants)
PBMC-SP	18–24 h	4	35
	48 h	3	26
PBMC-AQ	24 h	1	8
PBL/HUVEC	24 h	1	8
WB-AQ	24 h	6	54
	48 h	1	8
dWB-AQ	48 h	1	12
dWB-SP*	48 h	1	15

PBMC: Peripheral Blood Mononuclear Cells; PBL: peripheral blood leukocytes; HUVEC: human umbilical vein endothelial cells; WB: Whole Blood; dWB: diluted Whole Blood; SP: solid phase; AQ: Aqueous Phase; dWB-SP* corresponds to the bead coated method.



Vessillier, S., et al. (2020). "Development of the first reference antibody panel for qualification and validation of cytokine release assay platforms - Report of an international collaborative study." Cytokine X 2(4): 100042.

Can we develop a reference antibody panel to qualify and validate preclinical immunogenicity assays ?

Outline

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What biologics should compose the reference panel ?

Desired attributes:

- Homologs to clinically tested therapeutic biologics
- At least one strong positive control that will reproducibly trigger a response in diverse assays
  - ➢ **Anti-IL21R antibody** (Homolog to ATR-107; 76% ADA+)
- A negative and positive control antibodies targeting the same antigen (mimicking industry preclinical experience)
  - ➢ **Anti-PCSK9 antibody A** (Homolog to Evolocumab; <1% ADA+)
  - ➢ **Anti-PCSK9 antibody B** (Homolog to Bococizumab; 48% ADA+)

Discussed but not included for cost/feasibility consideration

- Immunogenic peptides

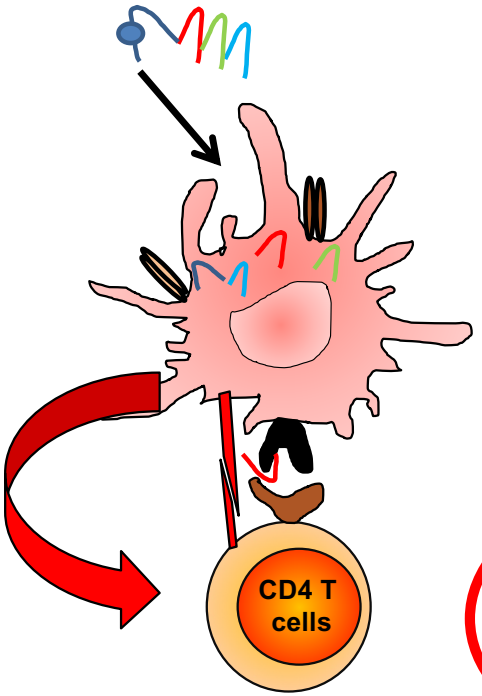
What preclinical assays to focus on first ?

INNATE IMMUNE RESPONSE

SIGNAL 2

DC/Monocyte Activation

- Upregulation of costimulation molecules (CD80,CD86, CD40) and activation markers (CD83)
- Production of inflammatory cytokines/chemokines



DC/Monocyte internalization

ADAPTIVE IMMUNE RESPONSE

SIGNAL 1

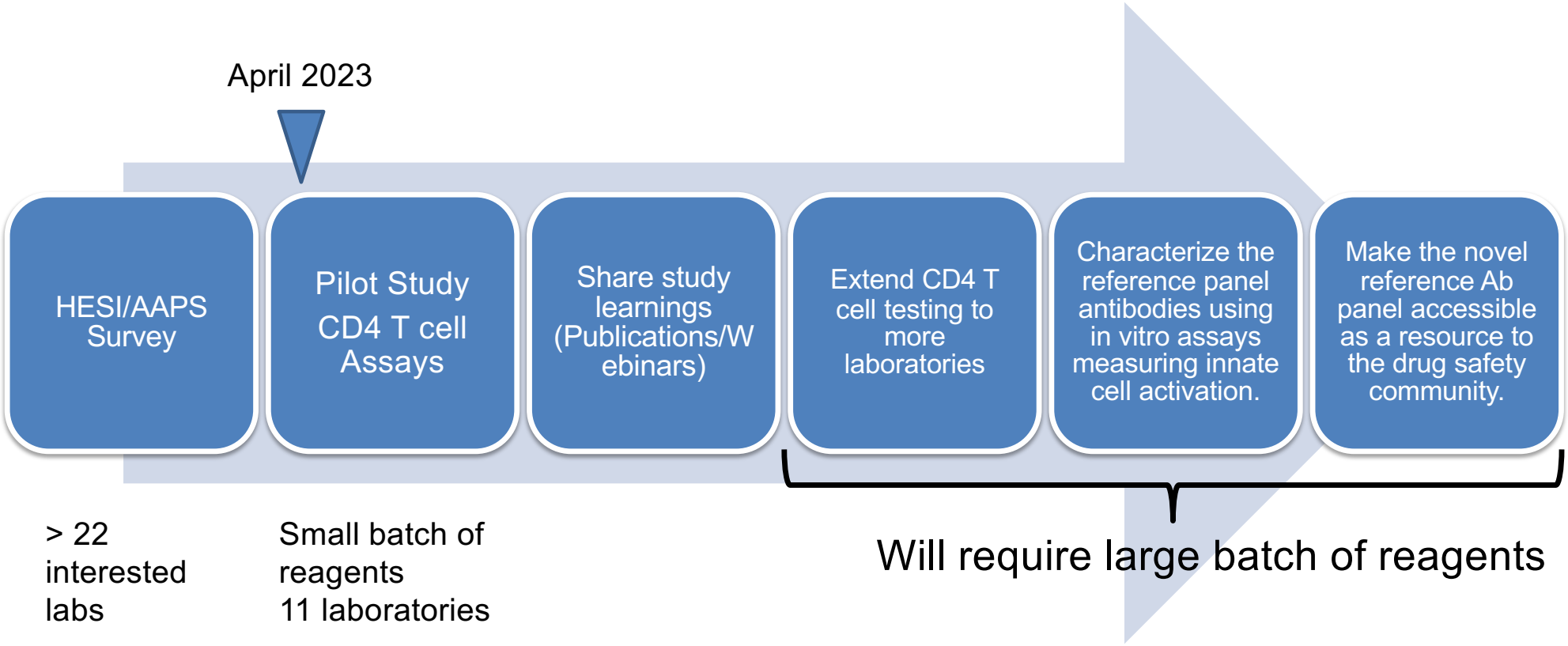
Identification of potential T cell epitopes

- MAPPS

CD4 T cell activation assays

Most frequently cited preclinical assay In AAPS survey

Reference Panel Development: Activities and Timeline



Pilot study - Characterize the reference panel antibodies using in vitro T cell activation assays

- **Goals:** to assess the activity/immunogenicity of the antibodies produced by Absolute Antibody and to the effect/impact of freeze-drying process on the activity/immunogenicity of the antibodies
- 11 participating laboratories
- Funded by HESI-ITC
- 250-500mg of the 3 reference panel antibodies produced by Absolute Antibodies
- Product Lyophilization at NIBSC and shipment to participating laboratories
    - ❑ Data collection from various T cell assays : Q2-Q3 2023
    - ❑ Data analysis and Manuscript writing : Q3-Q4 2023

Reference Panel Development Status

- 11 Laboratories participating to the pilot study (Abbvie, BMS, Eli Lilly, Epivax, FDA (x2), Genentech, ImmunXperts, Merck, Pfizer, Sanofi)

Assay Type	Incubation Time	Readout	Number of participants
PBMC Assay	7d	IFN-γ	4
PBMC Assay	7d	IL-2	2
PBMC Assay	2d	T cell activation marker	1
PBMC Assay (wo CD8)	7d	Proliferation (CFSE or EdU)	3
DC:T cell Assay	7d	CD4 T Cell Proliferation	2
DC:T cell Assay	7d	IFN-γ	2
DC:T cell Assay	7d	IL-2	1

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Lilly Flow-Based T cell Proliferation Assay

CTL Cellular Technology Limited

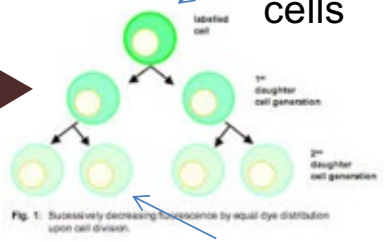
CFSE labeling

Non-proliferating cells

Cell Culture (7days)

+/- Biologics

HLA-typed PBMC (CD8+ Tcells are depleted)

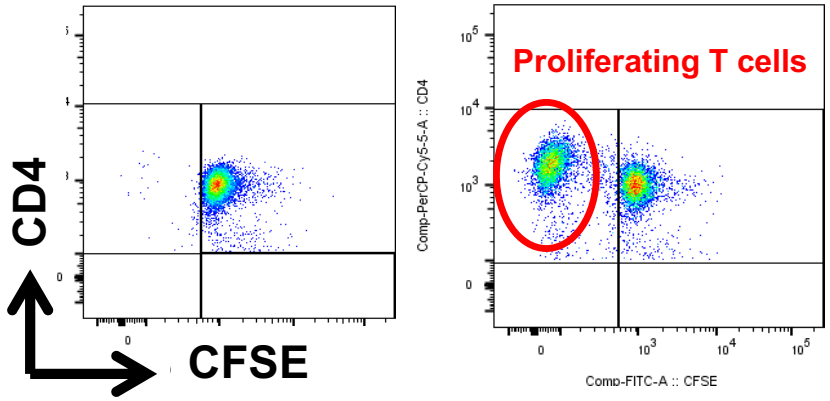


Proliferating Cells

Flow Cytometry

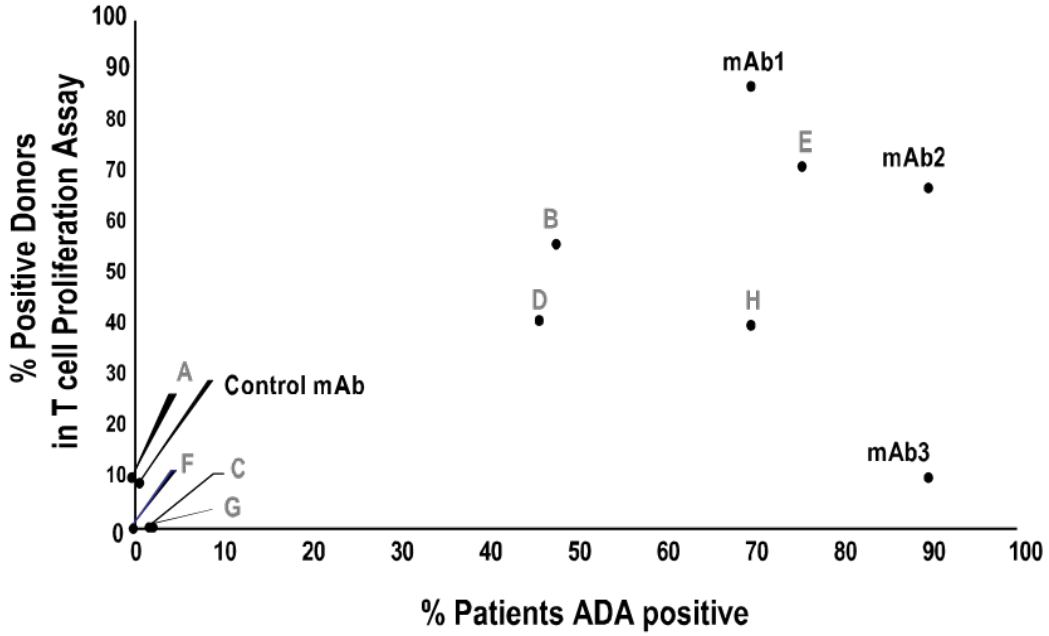
Media Only Control

Positive Control



Walsh et al mAb 2020

Previous relevant experience in PBMC assay with internal homologs



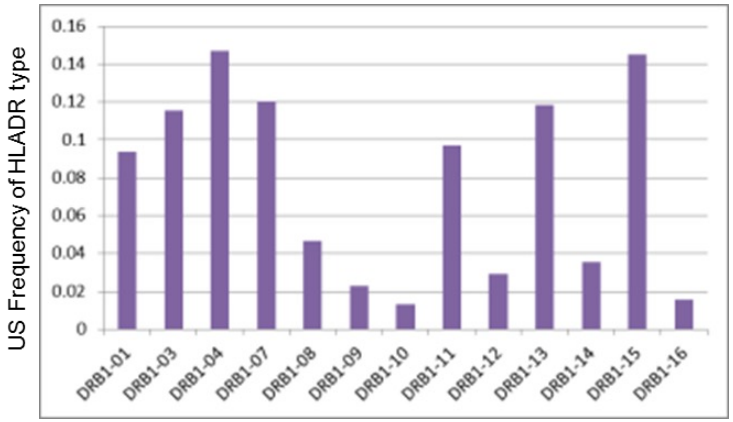
Label	Biologics	Description
A	Anti-PCSK9 A*	Evolocumab homolog
B	Anti-PCSK9 B*	Bococizumab homolog
C	Anti-IL4R A*	Dupilumab homolog
D	Anti-IL4R B*	AMG317 homolog
E	Anti-IL21R*	ATR-107 homolog
F	Pembrolizumab	Anti-PD-1 mAb
G	Tocilizumab	Anti-IL-6R mAb
H	GLP-1R agonist*	Lixisenatide homolog

Walsh et al mAb 2020

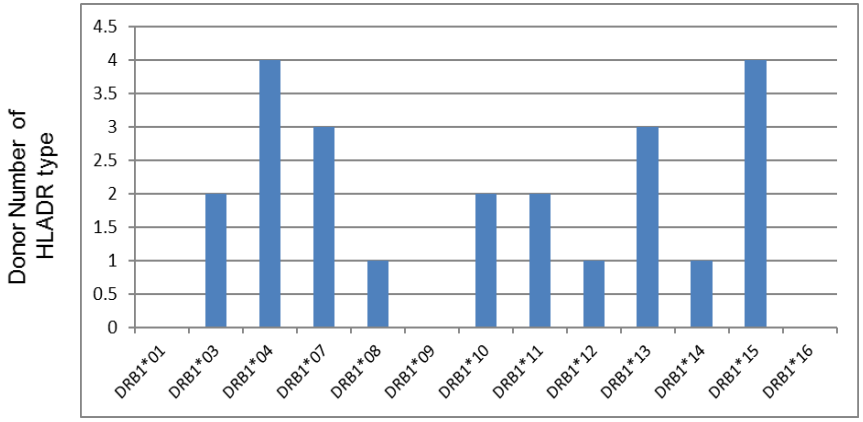
HLA-DR Allotype Expression in cohort used for T cell Proliferation Assessment of the Reference Panel

Donor	DRB1 allele 1	DRB1 allele 2	Ethnicity
1	*07:01	*14:06	Hispanic
2	*07:01	*15:01	Caucasian
3	*07:01	*13:02	AfricanAmerican/Hispanic
4	*04:07	*04:07	Hispanic
5	*08:04	*11:01	African/American
6	*04:02	*12:01	African/American
7	*03:01	*04:01	Caucasian
8	*13:02	*15:03	Hispanic
9	*04:01	*10:01	Caucasian
10	*03:01	*15:01	Hispanic
11	*10:01	*13:05	Caucasian
12	*11:01	*15:03	African/American

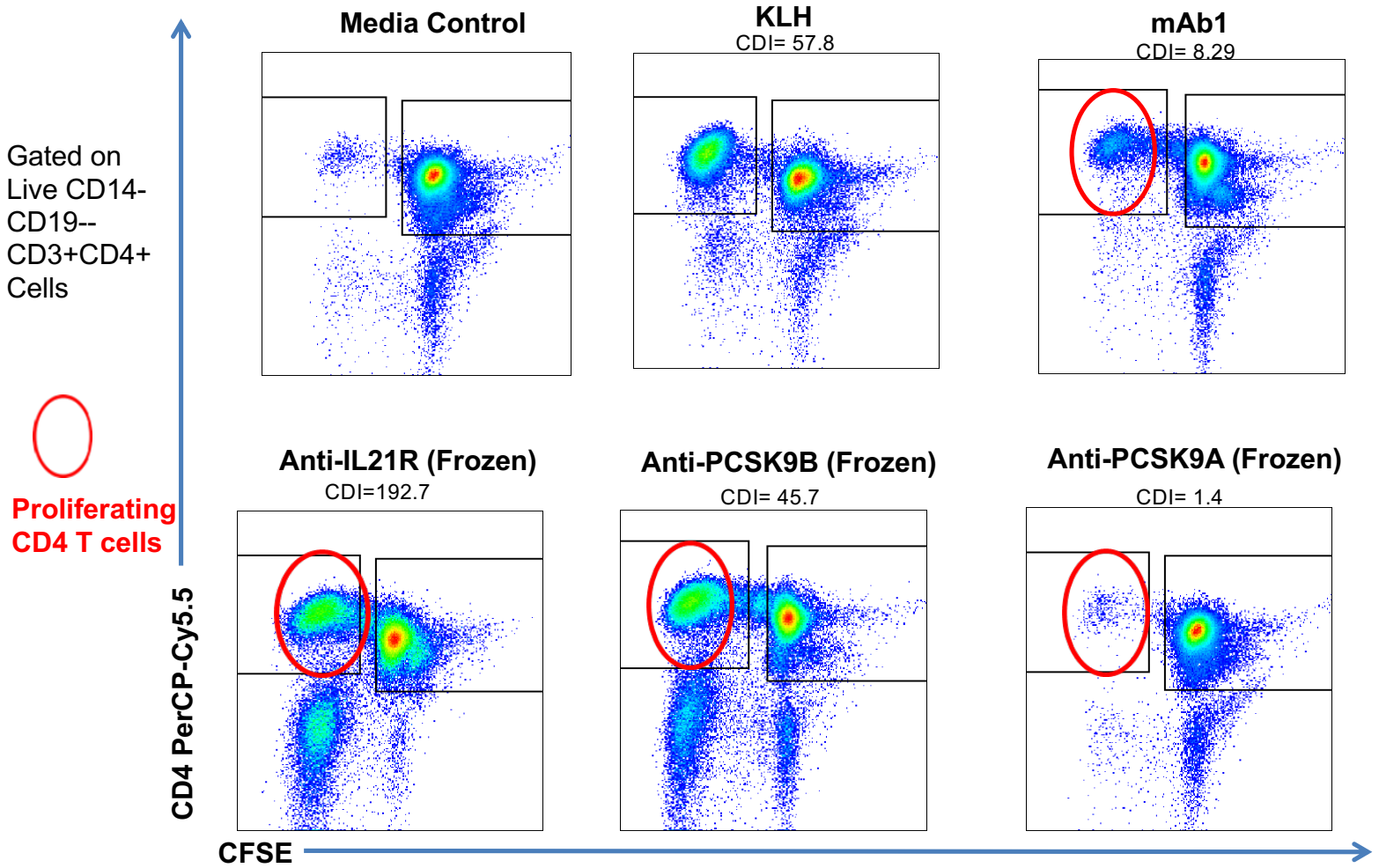
HLA-DR Haplotypes Expressed in US Populations



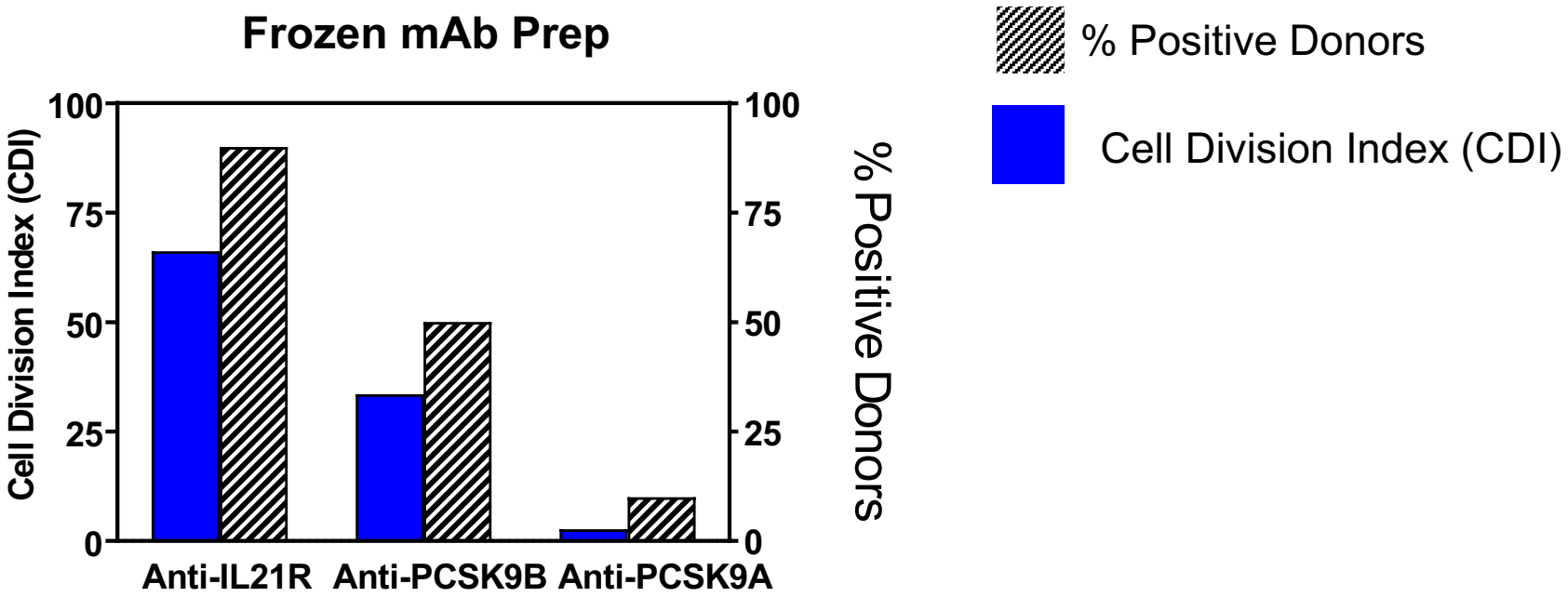
HLA-DR Haplotypes Expressed in This Study Cohort



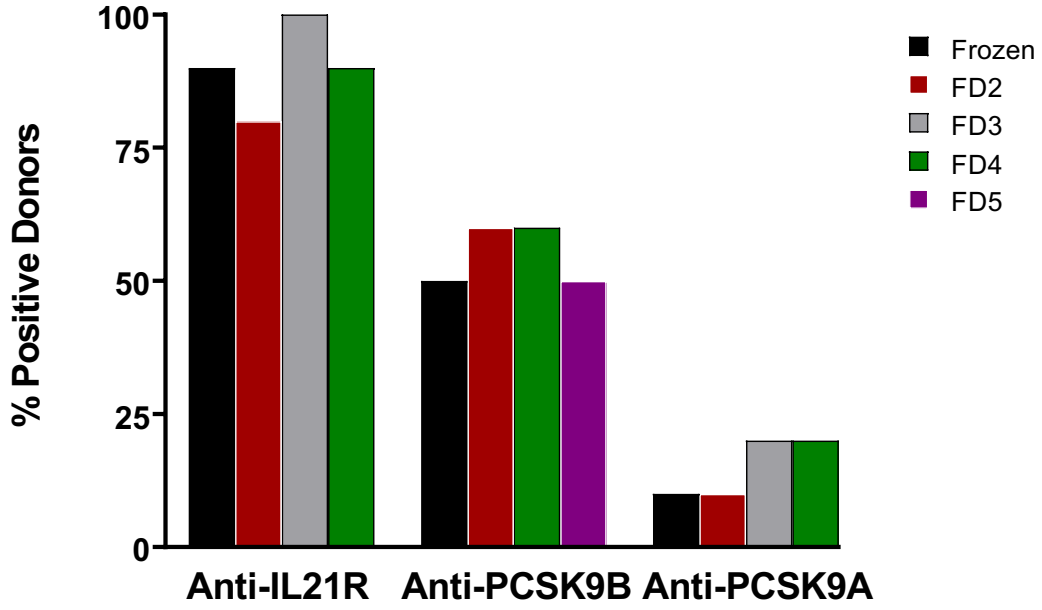
T Cell Proliferation Results with Reference Panel Antibodies
Representative Pseudocolor Flow Plots



T Cell Proliferation Results with Reference Panel Antibodies across 12 donors



No Impact of Lyophilization on CD4 T cell Proliferative Response to Reference Panel Antibodies



Summary

- There is no alignment on the methods used for assessing the immunogenicity risk.
- One of the challenges for the development and comparison of assay performance is the lack of availability of standard positive and negative control antibodies for use in assay qualification.
- The development of a reference panel of positive and negative control monoclonal antibodies will provide information on the reproducibility, robustness and potential limitations of preclinical assays developed to predict clinical immunogenicity.

Questions ?

Interested to join ?

Contacts

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Thank you