EIP
European Immunogenicity Platform

Standardization of in-Vitro Assays Update from the AAPS/HESI Collaboration

15TH OPEN SCIENTIFIC EIP SYMPOSIUM ON IMMUNOGENICITY OF BIOPHARMACEUTICALS

22ND — 24TH APRIL 2024

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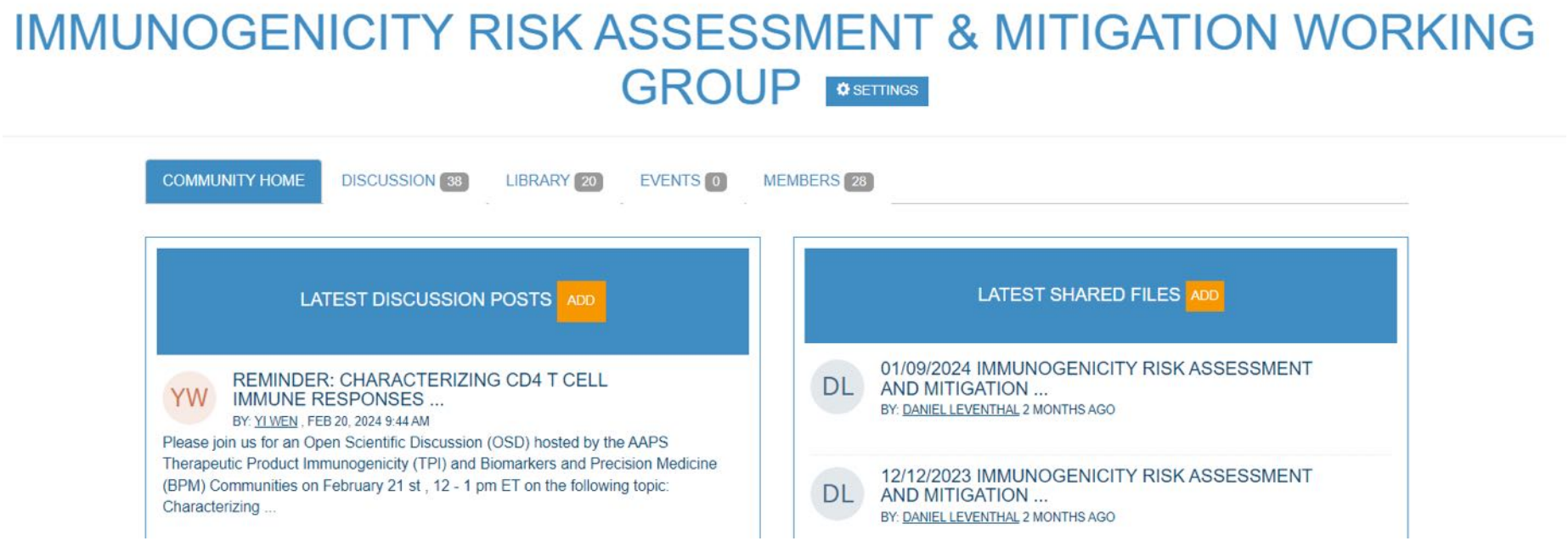
Background



Source: https://community.aaps.org/communities/community-home

Survey

AAPS J. 2023 Jun 2;25(4):55. doi: 10.1208/s12248-023-00820-7.

Survey Outcome on Immunogenicity Risk Assessment Tools for Biotherapeutics: an Insight into Consensus on Methods, Application, and Utility in Drug Development

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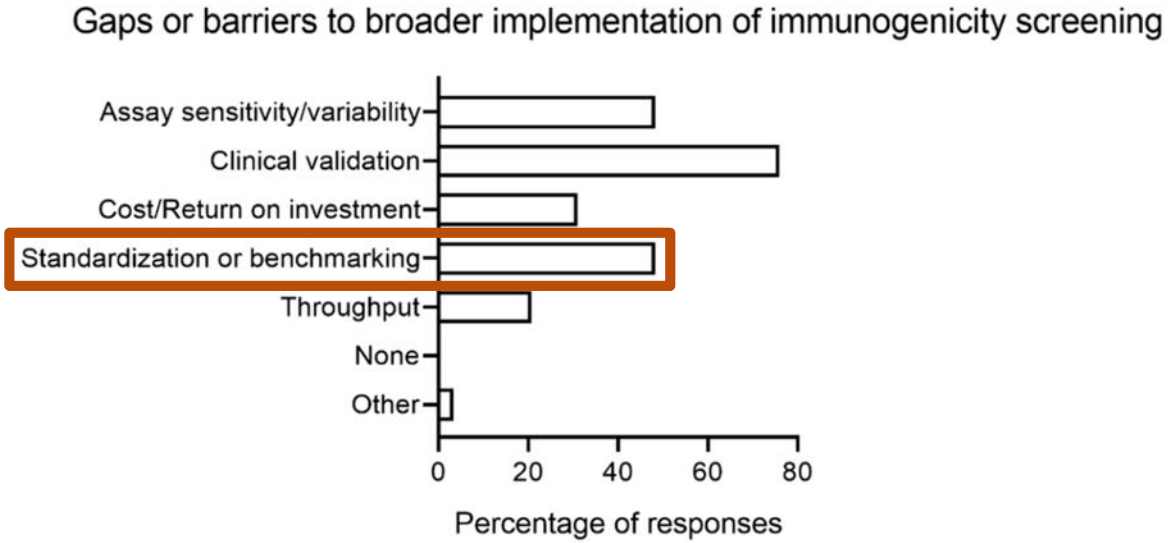
Survey

From: Survey Outcome on Immunogenicity Risk Assessment Tools for Biotherapeutics: an Insight into Consensus on Methods, Application, and Utility in Drug Development



Gaps or barriers to broader implementation of immunogenicity screening from the first survey conducted in 2016

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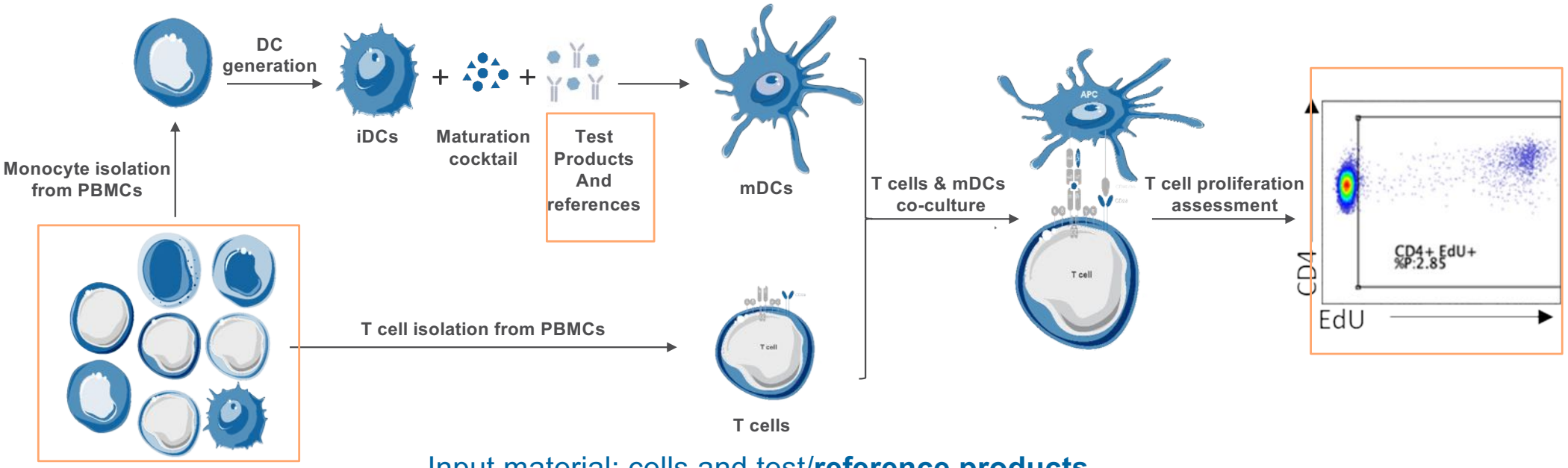
Exchange of information within the communities

- ✓ Varying results with **different batches** of **Bococizumab**: from **40 to 80%** of responding donors when tested in an in vitro screening
- ✓ Different '**versions**' **of ATR-107 available**
- ✓ Issues with KLH batches/suppliers: inhibition/toxicity observed
- ✓ ….

Standardisation of in vitro assays: where do we start?



Input material: cells and test/**reference products**
Method/protocol
Data analysis

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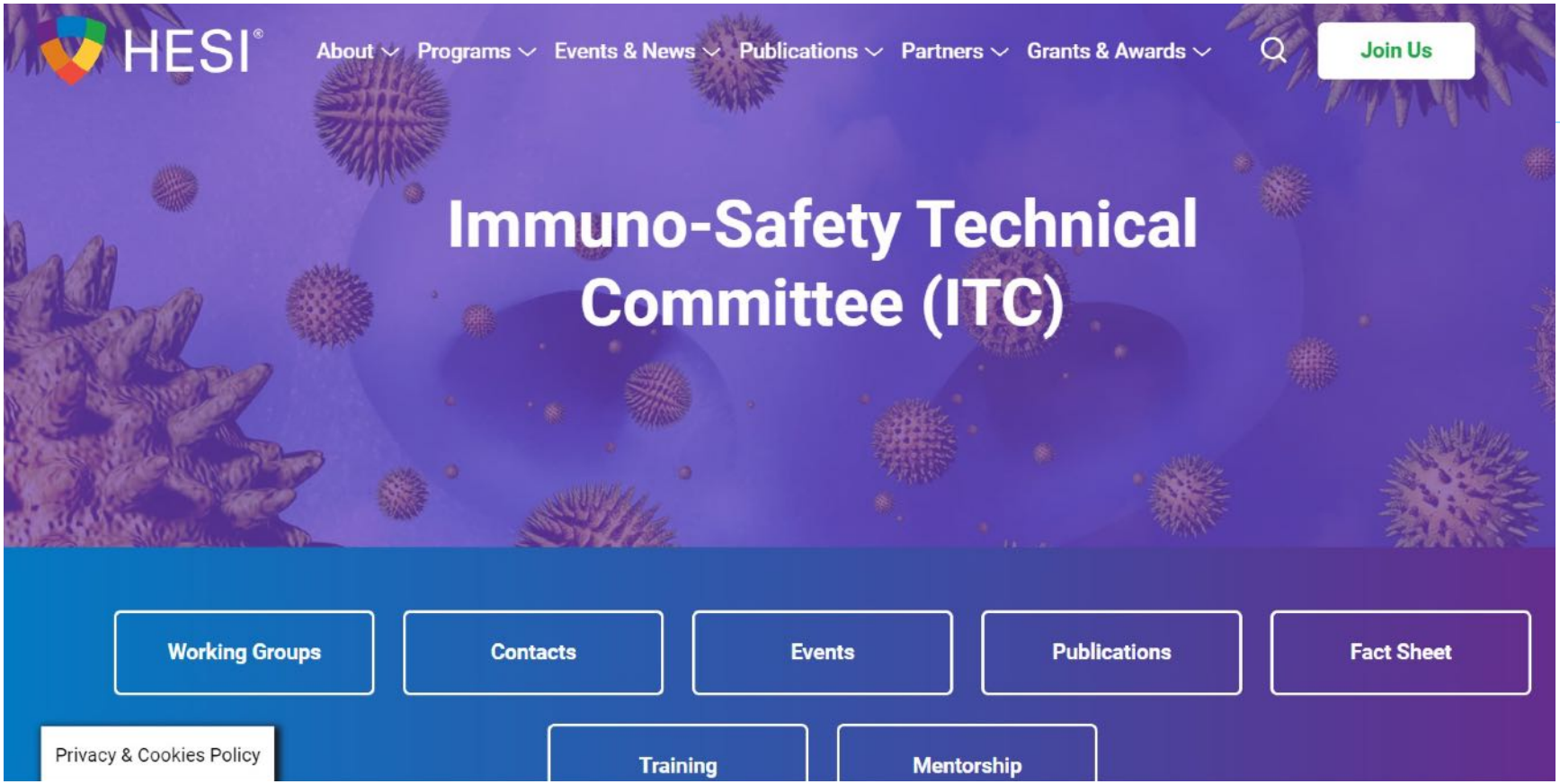

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HESI-ITC Method Development Workgroup

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

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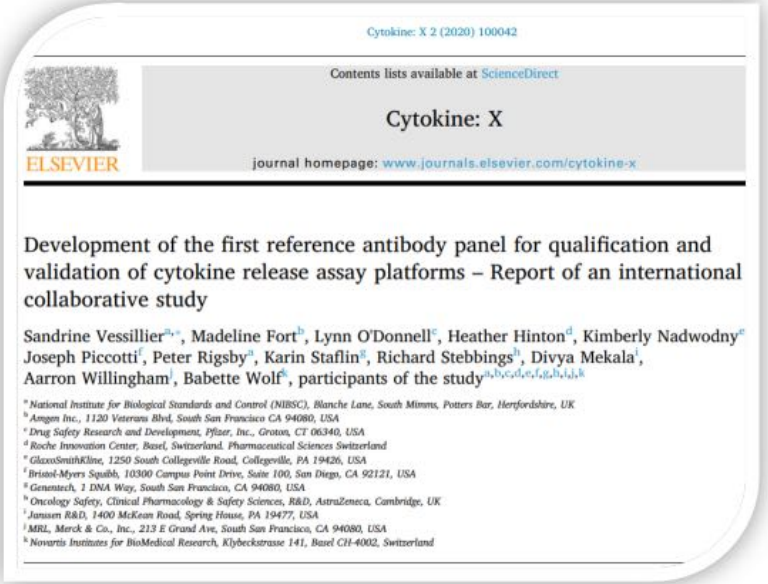
Cytokine Release Assay Reference Panel

- " 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

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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

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HESI/AAPS Reference Panel

- ✓ *Anti-IL21R antibody* (Homolog to ATR-107; 76% ADA+)*
- ✓ Anti-PCSK9 antibody (Homolog to Evolocumab; <1% ADA+)
- ✓ Anti-PCSK9 antibody (Homolog to Bococizumab; 48% ADA+)

Antibodies produced by 3rd party, 1st lyophilization done by NIBSC
Pre-pilot testing performed by one lab
2nd batch lyophilized, pilot testing performed by 11 labs

**First test results of the anti-IL21R antibodies showed an increased level of endotoxin, so it was decided to not include the data/sample in the pilot run*.

Show the feasibility of lyophilized material for in vitro immunogenicity screening/comparison with frozen soluble test material.

Results in vitro testing

- ✓ All participating labs performed their in house analysis and the majority presented their data/conclusions during HESI/AAPS meetings
- ✓ All particpants were requested to upload their raw data for uniform statistical analysis
- ✓ Raw data available from 5 labs, further raw data collection ongoing

Preliminary analysis 5 data sets

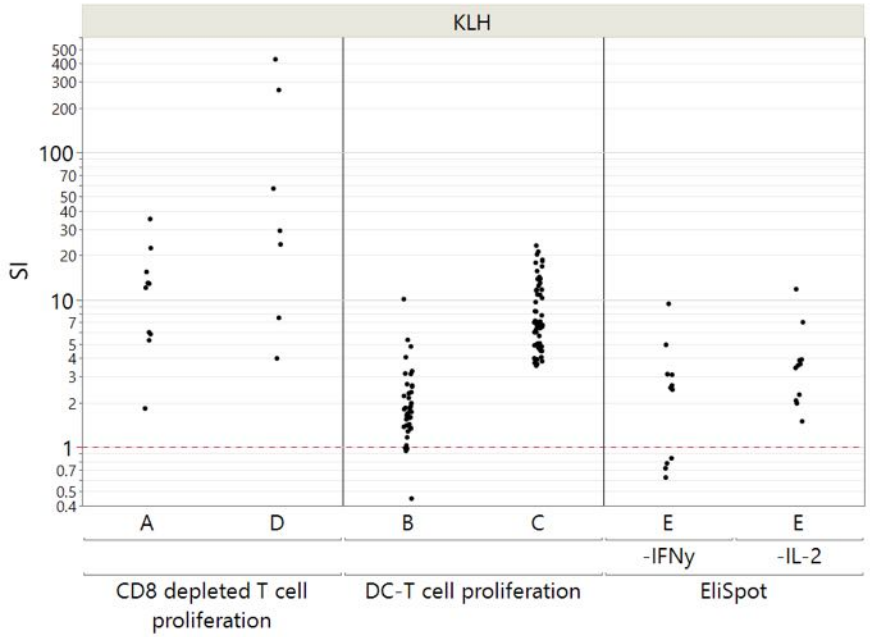
Assay*	Study	# Donors	# Replicates
CD8 depleted T cell proliferation	A	10	3 (6 for neg ctrl)
CD8 depleted T cell proliferation	D	7	3
DC-T cell proliferation	B	40**	6
DC-T cell proliferation	C	53	8
EliSpot	E	11	6

*type of assay, not necessarily same protocol
**not for all conditions

Preliminary analysis 5 data sets

- All participants report the ratio of response in test condition/response in reference condition (stimulation index =SI) as main outcome per donor
- **Reported % positive donors by participants** is determined either based on:
  - **Empirical threshold: SI>2**
  - **Statistical testing**:
    - ✓ **T-test evaluating** if *difference* in test versus reference condition is significantly different from 0
    - ✓ **Non-parametric equivalence test after logarithmic transformation** evaluating if SI is significantly below 2
- For this **presentation raw data was uniformly re-analyzed across the studies**:
  - **SI** was calculated per donor (based on geometric mean in test and reference condition)
  - **Overall geometric mean SI** over all donors was calculated
  - The **% of donors with SI>2 calculated**
  - The % of donors with an SI significantly higher than 1 was calculated by t-tests on logarithmically transformed results
    - ✓ To not violate test assumptions: **results do not follow a normal distribution without transformation**
    - ✓ Allows to directly test the outcome of interest (=SI): difference on log scale = ratio on natural scale

Results positive control KLH

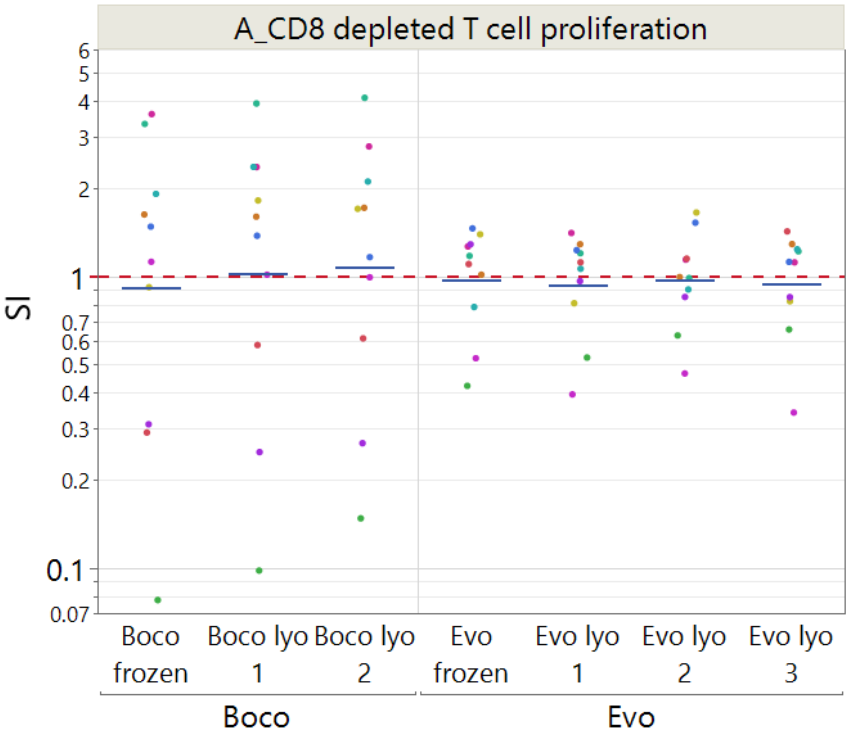


Stimulation index (SI) = geometric mean stimulated condition/geometric mean control

All studies included a KLH control, with positive responses observed across most donors in all studies, with varying SI.

⇒ SI across the studies cannot be directly compared

Study A



Note the **larger range** in response to **Bococizumab** compared to Evolocumab, in the 2 directions (< and >1).

Because of this, relative to the inter-donor variation within compound, there is no consistent difference in response between compounds.

There is a **clear trend for Bococizumab to result in higher % of positive responses compared to Evolocumab**, with **consistent** results across **lyophilized and frozen** batches.

	Geomean SI		% SI>2		% p<0.05	
Batch	Boco	Evo	Boco	Evo	Boco	Evo
frozen	0.91	0.97	20%	0%	50%	0%
lyo 1	1.03	0.94	30%	0%	40%	0%
lyo 2	1.07	0.97	30%	0%	40%	10%
lyo 3		0.94		0%		10%

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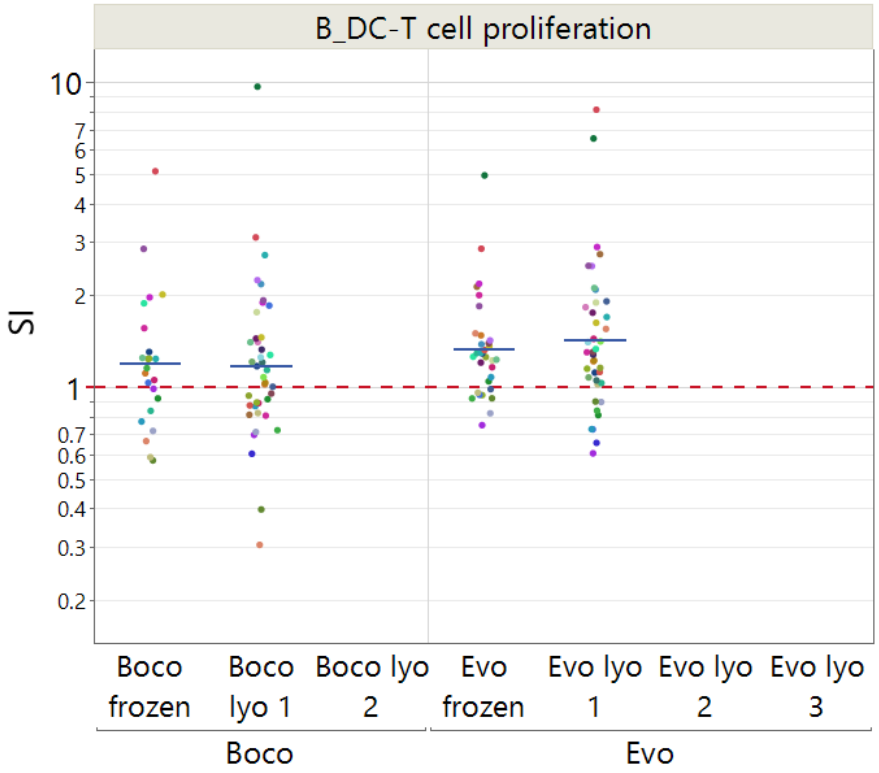
Study B



Bococizumab and Evolocumab result in geometric mean SI and % of positive donors in the **same range**, without any tendency for Bococizumab to result in higher responses.

Results are relatively **consistent across frozen and lyophilized form**, although there is a **tendency for lyophilized Evocolumab** to result in **slightly higher** response rate than frozen Evocolumab.

	Geomean SI		% SI>2		% p<0.05	
Batch	Boco	Evo	Boco	Evo	Boco	Evo
frozen	1.20	1.33	13%	16%	22%	19%
lyo 1	1.17	1.43	13%	20%	23%	33%

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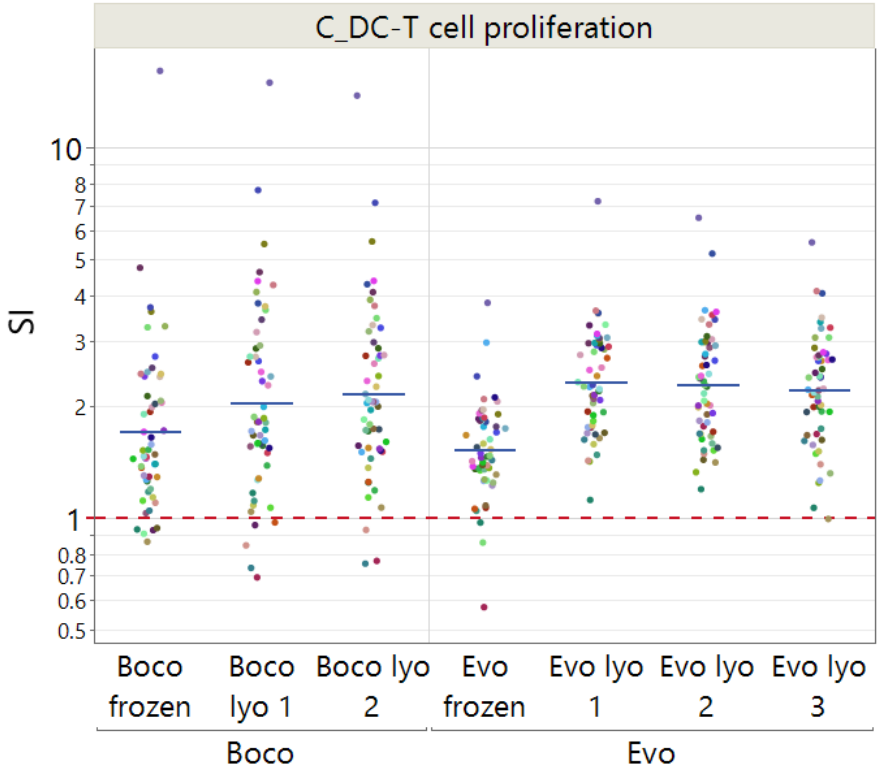
Study C



Frozen **Bococizumab results in slightly higher overall geometric mean** than frozen **Evolocumab**, with also a higher % of donors showing SI>2.

There is a however a clear tendency for **lyophilized batches** of **Bococizumab and especially Evolocumab** to result in **higher** overall geometric mean SI and higher % of positive donors. This is consistently present across the different batches of lyophilized products.

	Geomean SI		% SI>2		% p<0.05	
Batch	Boco	Evo	Boco	Evo	Boco	Evo
frozen	1.72	1.53	32%	11%	64%	62%
lyo 1	2.05	2.33	42%	66%	70%	92%
lyo 2	2.16	2.29	55%	62%	81%	92%
lyo 3		2.23		64%		89%

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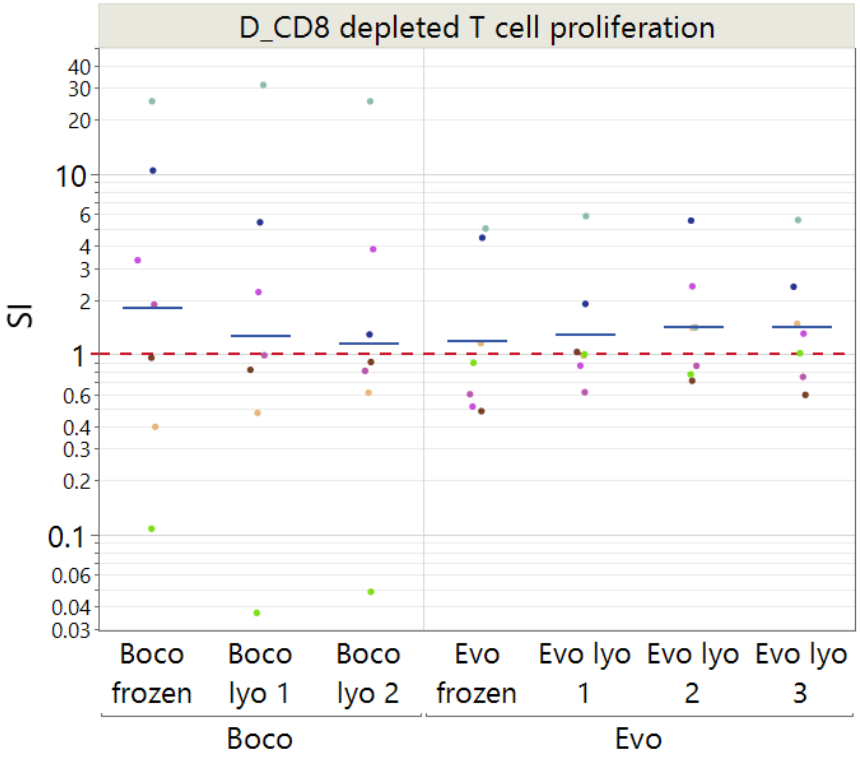
Study D



Note, similar to the other CD8 depleted assay, a wide range of response for Bococizumab, with also SI well <1 observed in one donor.

There is a **tendency for higher response to frozen Bococizumab** compared to frozen **Evolocumab**, but this is not consistently present across all lyophilized batches. Note that with the low number of donors, the difference of 43% vs 29% donors with SI>2 is only 1 donor difference. The inability to consistently distinguish Bococizumab from Evolocumab across lyophilized batches is likely explained by variability of response and a low number of donors rather than a true effect of lyophilization.

	Geomean SI		% SI>2		% p<0.05	
Batch	Boco	Evo	Boco	Evo	Boco	Evo
frozen	1.83	1.19	43%	29%	29%	29%
lyo 1	1.27	1.30	43%	14%	29%	14%
lyo 2	1.16	1.44	29%	29%	14%	29%
lyo 3		1.42		29%		29%

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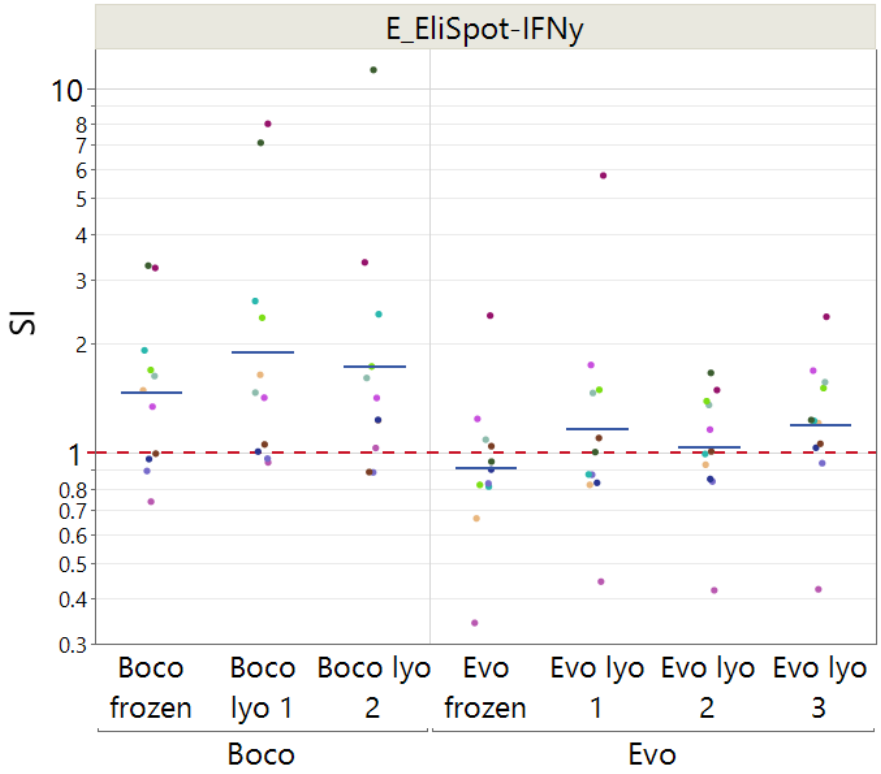
Study E



All batches of **Bococizumab** (frozen and lyophilized) result in consistently higher geometric mean SI and % positive donors than all batches of Evolocumab.

There is a tendency for **higher** geometric mean SI and higher % of positive donors for **lyophilized versus frozen**.

	Geomean SI		% SI>2		% p<0.05	
Batch	Boco	Evo	Boco	Evo	Boco	Evo
frozen	1.47	0.91	18%	9%	55%	9%
lyo 1	1.90	1.17	36%	9%	55%	27%
lyo 2	1.74	1.04	27%	0%	55%	0%
lyo 3		1.20		9%		36%

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Conclusions: Lyophilized versus frozen

- Most studies do not show systematic differences between **lyophilized** or **frozen** products:
  - One study with 53 donors (DC-T cell assay) shows **very consistently higher responses for all lyophilized** batches compared to frozen product, to an extent that differences between compounds are not maintained.
  - Another study (EliSpot) shows a **similar tendency for higher responses for lyophilized** versus **frozen** product, but still across batches (frozen and lyophilized) the differences between compounds remains present.
  - The 3 other studies **do not show any or only a minimal difference between frozen and lyophilized products.**

Next steps

- Complete statistical analysis
- Compare observations/findings within the different assay groups and assess the potential time of storage effect
- Lessons learned (blind testing, limit the testing window, …)
- Already dreaming of the next steps (using one source of PBMC, …)
- Continue to look for funding

A BIG THANK YOU!

Participants name	Organization
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