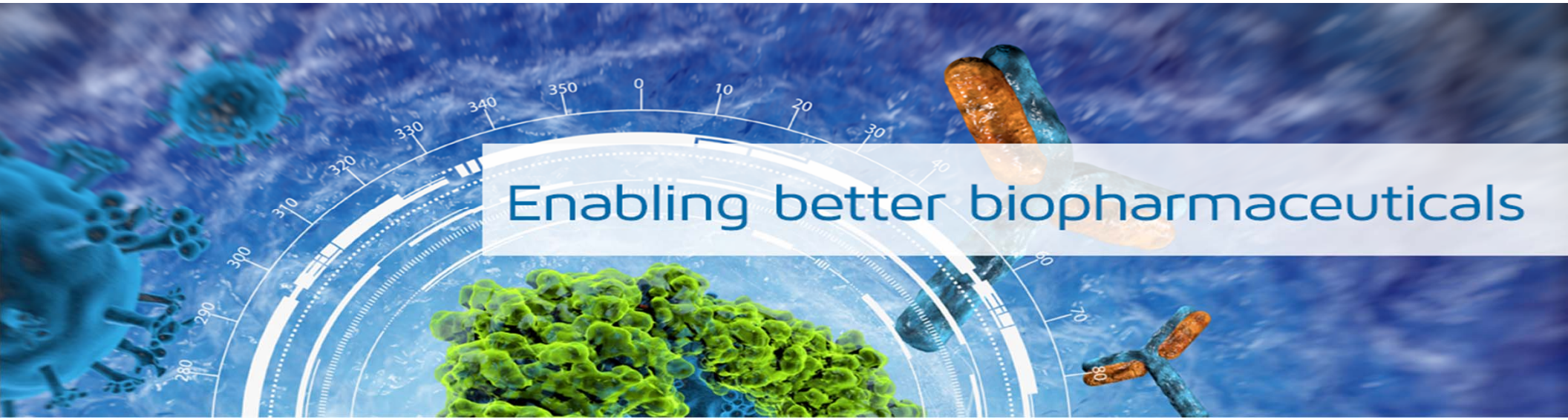


Pre-clinical Immunogenicity Risk Management for Biologics and Aggregates

Mark Fogg PhD
Head of Immunology - Abzena

2017 PDA Pre-conference Workshop
Impact of Pre-filled Syringe Packaging Components on Biopharmaceuticals
6th November 2017

ABZENA
Enabling better biopharmaceuticals

Outline

- Introduction
  - Anti Drug Antibodies - ADA
- Technologies
  - PBMC based assays
  - DC:T cell assays
  - TCEM
  - MAPPs
- Use of assays for immunogenicity risk evaluation of aggregates
- Summary

Pegloticase: ADA and Efficacy

Lipsky *et al. Arthritis Research & Therapy* 2014, **16**:R60
http://arthritis-research.com/content/16/2/R60

arthritis research&therapy

RESEARCH ARTICLE Open Access

Pegloticase immunogenicity: the relationship between efficacy and antibody development in patients treated for refractory chronic gout

Peter E Lipsky[1*], Leonard H Calabrese[2], Arthur Kavanaugh[3], John S Sundy[4], David Wright[5], Marsha Wolfson[6] and Michael A Becker[7]

Abstract

Introduction: The efficacy of pegloticase, a polyethylene glycol (PEG)-conjugated mammalian recombinant uricase, approved for chronic refractory gout, can be limited by the development of antibodies (Ab). Analyses from 2 replicate, 6-month, randomized controlled trials were performed to characterize Ab responses to pegloticase.

Methods: Anti-pegloticase, anti-PEG, and anti-uricase Ab were determined by validated enzyme-linked immunosorbent assays. Ab titers were analyzed for possible relationships with serum pegloticase concentrations, serum uric acid (sUA) lowering, and risk of infusion reactions (IRs).

Results: Sixty-nine (41%) of 169 patients receiving pegloticase developed high titer anti-pegloticase Ab (> 1:2430) and 40% (67/169) developed anti-PEG Ab; 1 patient receiving placebo developed high titer anti-pegloticase Ab. Only 14% (24/169) of patients developed anti-uricase Ab, usually at low titer. In responders, patients showing sustained UA lowering, mean anti-pegloticase titers at week 25 (1:837 ± 1687 with biweekly and 1:2025 ± 4506 with monthly dosing) were markedly lower than in nonresponders (1:34,528 ± 42,228 and 1:89,658 ± 297,797, respectively). Nonresponder status was associated with reduced serum pegloticase concentrations. Baseline anti-pegloticase Ab, evident in 15% (31/212) of patients, did not predict subsequent loss of urate-lowering response. Loss of sUA response preceded IRs in 44 of 56 (79%) pegloticase-treated patients.

Conclusions: Loss of responsiveness to pegloticase is associated with the development of high titer anti-pegloticase Ab that increase clearance of pegloticase and are associated with a loss of the sUA lowering effect and increased IR risk. Pre-infusion sUA can be used as a surrogate for the presence of deleterious anti-pegloticase Ab.

Trial registration: NCT00325195. Registered 10 May 2006, NCT01356498. Registered 27 October 2008.

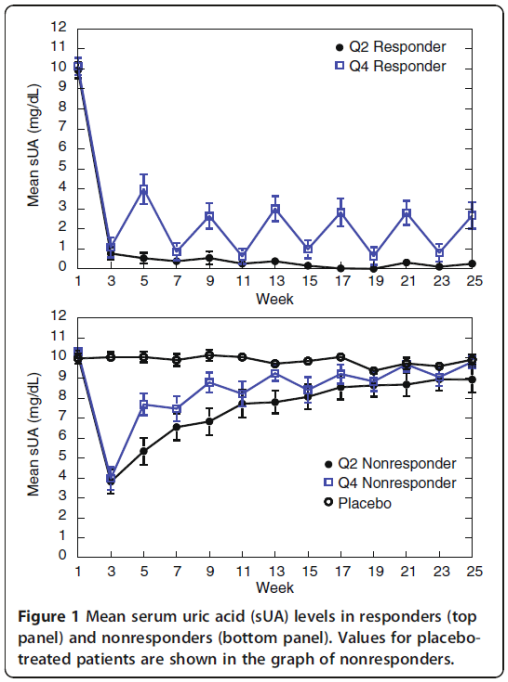


Figure 1 Mean serum uric acid (sUA) levels in responders (top panel) and nonresponders (bottom panel). Values for placebo-treated patients are shown in the graph of nonresponders.

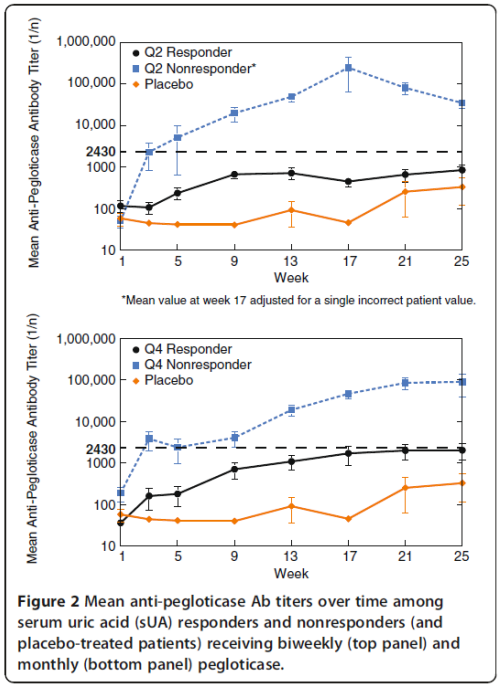


Figure 2 Mean anti-pegloticase Ab titers over time among serum uric acid (sUA) responders and nonresponders (and placebo-treated patients) receiving biweekly (top panel) and monthly (bottom panel) pegloticase.

ABZENA
Enabling better biopharmaceuticals

Anti-TNFs: ADA, Efficacy and Switching

ORIGINAL CONTRIBUTION

Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up

Geertje M. Bartelds, MD
Charlotte L. M. Krieckaert, MD
Michael T. Nurmohamed, MD, PhD
Pauline A. van Schouwenburg, MSc
Willem F. Lems, MD, PhD
Jos W. R. Twisk, PhD
Ben A. C. Dijkmans, MD, PhD
Lucien Aarden, PhD
Gerrit Jan Wolbink, MD, PhD

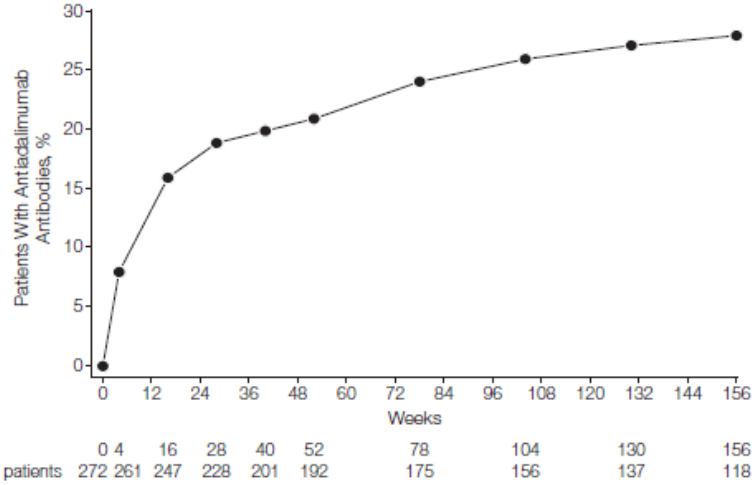
Context Short-term data on the immunogenicity of monoclonal antibodies showed associations between the development of antidrug antibodies and diminished serum drug levels, and a diminished treatment response. Little is known about the clinical relevance of antidrug antibodies against these drugs during long-term follow-up.

Objective To examine the course of antidrug antibody formation against fully human monoclonal antibody adalimumab and its clinical relevance during long-term (3-year) follow-up of patients with rheumatoid arthritis (RA).

Design, Setting, and Patients Prospective cohort study February 2004-September 2008; end of follow-up was September 2010. All 272 patients were diagnosed with RA and started treatment with adalimumab in an outpatient clinic.

Main Outcome Measures Disease activity was monitored and trough serum samples were obtained at baseline and 8 time points to 156 weeks. Serum adalimumab con-

Figure 1. Percentage of Antiadalimumab Development Over Time



- 21% stopped – treatment failure
- 11% SAEs
- 4% SAEs & treatment failure
- BUT 67% of total ADAs during the 1st 28 weeks

Development of secondary non-responsiveness due to ADAs can lead to switching to an alternative anti-TNF

ABZENA
Enabling better biopharmaceuticals

Fully Human Abs, FIH Study Halted Due to ADA

Clinical & Experimental Immunology
The Journal of Translational Immunology
Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/cei.12711

Contribution of enhanced engagement of antigen presentation machinery to the clinical immunogenicity of a human interleukin (IL)-21 receptor-blocking therapeutic antibody

L. Xue,* T. Hickling,* R. Song,† J. Nowak‡ and B. Rup*
*Pharmacokinetics, Dynamics and Metabolism-NBE, †Drug Safety R & D, and ‡Clinical Pharmacology, Pfizer Inc., Andover, MA, USA

Summary

Reliable risk assessment for biotherapeutics requires accurate evaluation of risk factors associated with immunogenicity. Immunogenicity risk assessment tools were developed and applied to investigate the

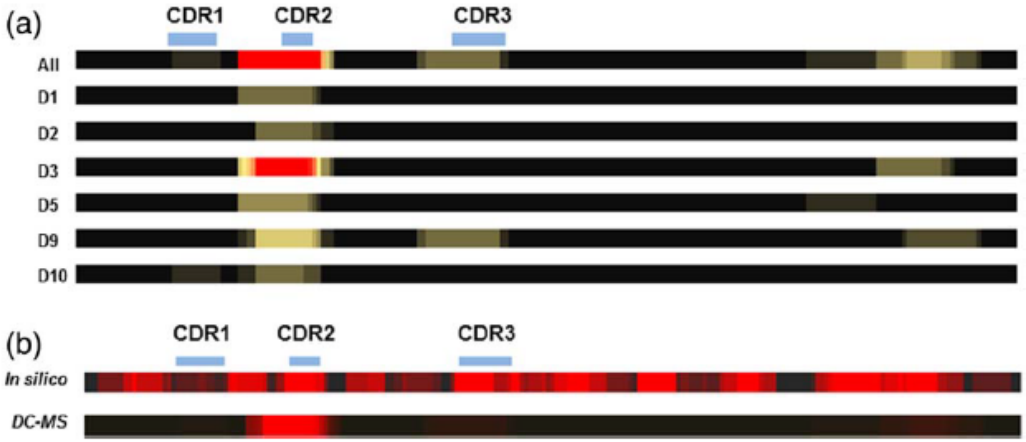


Table 2. Donor response frequency based upon %activated dendritic cell (DC) populations.

	%Cells ≥ 50% increase*			%Cells ≥ 100% increase*		
%Responders	KLH	ATR-107	PF-1	KLH	ATR-107	PF-1
%HLA-DR^{hi} CD11c^{hi}	89	64	36	78	64	18
%CD80^{hi} CD11c^{hi}	89	55	9	78	36	0
%OX40L^{hi} CD11c^{hi}	100	45	9	89	18	0
%CD86^{hi} CD11c^{hi}	100	73	9	100	73	0
%CD83^{hi} CD11c^{hi}	100	73	9	89	64	9
%CD40^{hi} CD11c^{hi}	100	64	18	100	45	9
%ICOSL^{hi} CD11c^{hi}	11	18	18	0	0	18
%PD-L1^{hi} CD11c^{hi}	100	82	0	100	73	0
%CCR7^{hi} CD11c^{hi}	78	45	9	67	27	0

- 76% of HVs ADA+ after a single dose
- Enhanced activation of DCs
- Preferential trafficking to late endosomes & association with HLA-DR
- MAPPs identifies epitope in CDR2

ABZENA
Enabling better biopharmaceuticals

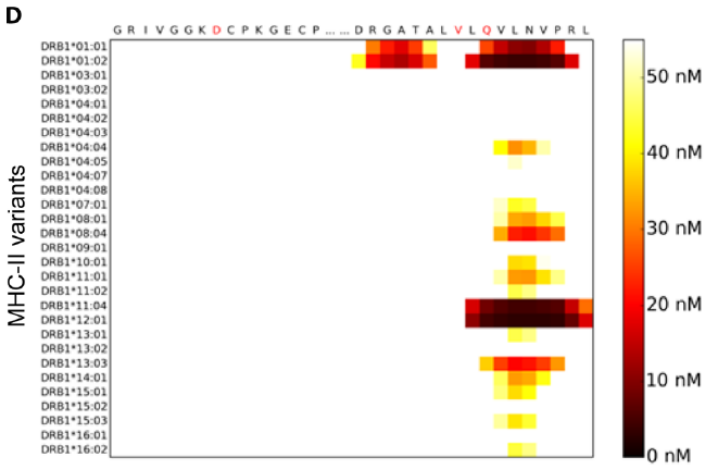
FVIIa, Discontinued After PhIII Due to ADA

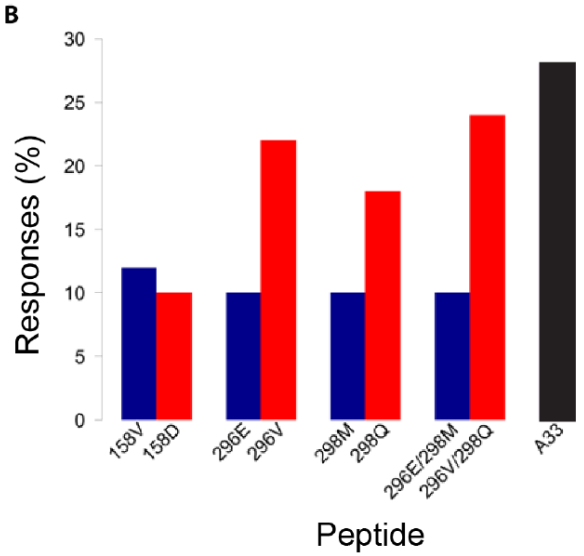
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

ANTI-DRUG ANTIBODIES

Post hoc assessment of the immunogenicity of bioengineered factor VIIa demonstrates the use of preclinical tools

Kasper Lamberth,[1]* Stine Louise Reedtz-Runge,[1] Jonathan Simon,[2] Ksenia Klementyeva,[2] Gouri Shankar Pandey,[2] Søren Berg Padkjær,[1] Véronique Pascal,[1] Ileana R. León,[1] Charlotte Nini Gudme,[1] Søren Buus,[3] Zuben E. Sauna[2]*





- Bioengineered analog of rFVIIa, vatreptacog alfa
- 99% sequence identity
- Discontinued in late stage clinical trials
- In silico tools identified a high affinity binding neoepitope
- E296v/M298Q substitutions increase T cell responses in naïve HD PMBC

Lamberth et al (2017) Sc Trans Med 9: 1286

ABZENA
Enabling better biopharmaceuticals

Erythropoietin: PRCA, ADAs and Safety

DISEASE OF THE MONTH
EBERHARD RITZ, FEATURE EDITOR

J Am Soc Nephrol 15: 398–406, 2004

Anti-Erythropoietin Antibodies and Pure Red Cell Aplasia

JEROME ROSSERT,* NICOLE CASADEVALL,† AND KAI-UWE ECKARDT,‡
*Department of Nephrology, Tenon hospital (AP-HP) and Pierre and Marie Curie University, Paris, France; †Department of Hematology, Hôtel Dieu (AP-HP), Paris, France; and ‡Department of Nephrology and Medical Intensive Care, Charité, Campus Virchow Klinikum, Berlin, Germany

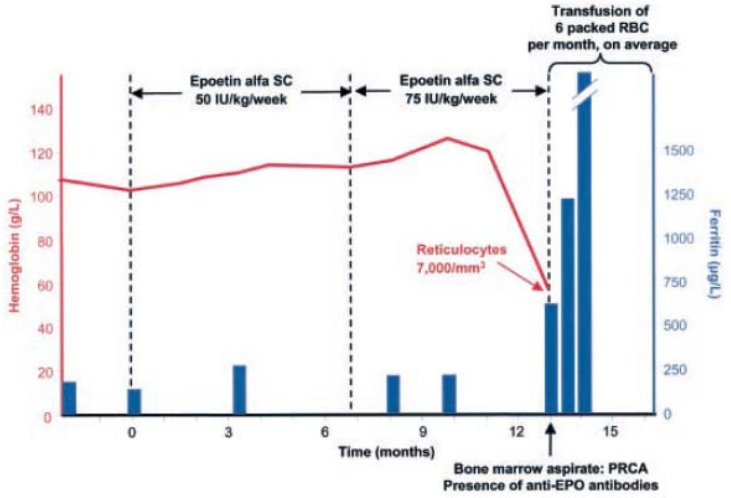


Figure 2. Schematic representation of a typical case of epoetin-induced PRCA. Red line: hemoglobin levels. Blue bars: ferritin levels.

- Change in route
- Change in formulation
- Soluble tungsten in the syringes
- Cross reactive, life threatening ADAs to a non-redundant hormone

ABZENA
Enabling better biopharmaceuticals

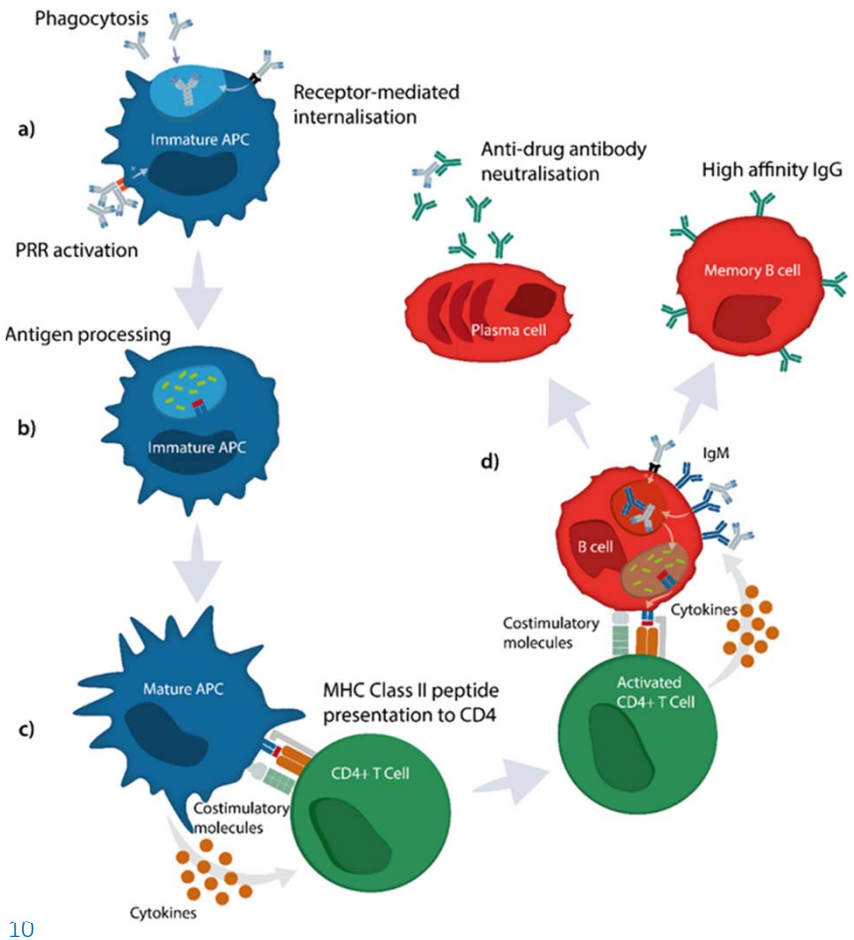
Immunogenicity to Therapeutic Proteins: a Broad Range of Consequences

- ADAs may be benign with no known clinical consequence
- ADAs can impact efficacy through altering PK (reduced exposure to the drug)
  - Switching to a competitor product (if available, anti-TNFs)
- Overt immunogenicity can halt development programs early (PhI)
- Worse, ADAs can halt development programs late (PhIII)
- ADAs can impact safety. Higher risk if ADAs cross react with a non-redundant endogenous counterpart (e.g. epo).
- Evidence for aggregation enhancing clinical immunogenicity risk

Immunogenicity: Main Factors

Associated with Product	Not Associated with Product	Associated with Patient
Self or non-self	Route of administration	Haplotype
B and T cell epitopes	Indication – chronic or acute disease	Tolerance to protein
Formulation/ Impurities/Aggregates	Pharmacokinetics	Immunosuppression
Post-translational modifications	Target – cellular or Soluble	

Role of T Cell Epitopes in a B Cell Response to a Biotherapeutic



- Linear peptides derived from the protein therapeutic during antigen-processing form complexes with MHC class II and activate T cells
- T cell help (from CD4+ T cells) is essential to development of high affinity, isotype switched (eg IgG) anti-therapeutic antibody responses
- Anti-therapeutic antibody responses can:
  - neutralise the activity of the protein therapeutic
  - reduce half-life by enhancing clearance
  - result in allergic reactions
  - cross-react with endogenous counterparts to result in 'autoimmune'-like reactions

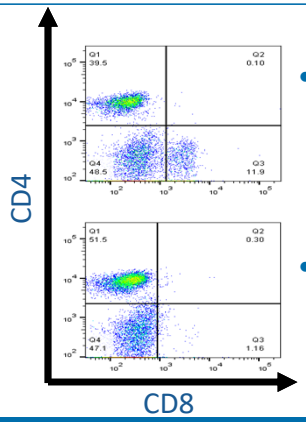
ABZENA
Enabling better biopharmaceuticals

Blood Processing for T cell Assays

Blood Processing

- 80-100 donors per week
- UK and Europe
- Tissue bank with >4500 donors
- Human Tissue Authority licenced facility
- Audited by HTA and clients

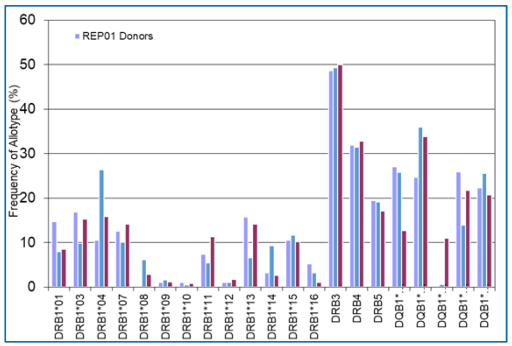
CD8+ Depletion



- For immunogenicity risk concentrate on MHC class II restricted responses
- Also donors banked with CD8+ T cells

HLA Distribution

- 50 donor cohort ensures proportional representation of HLA-DR/DQ allotypes in European/North American populations

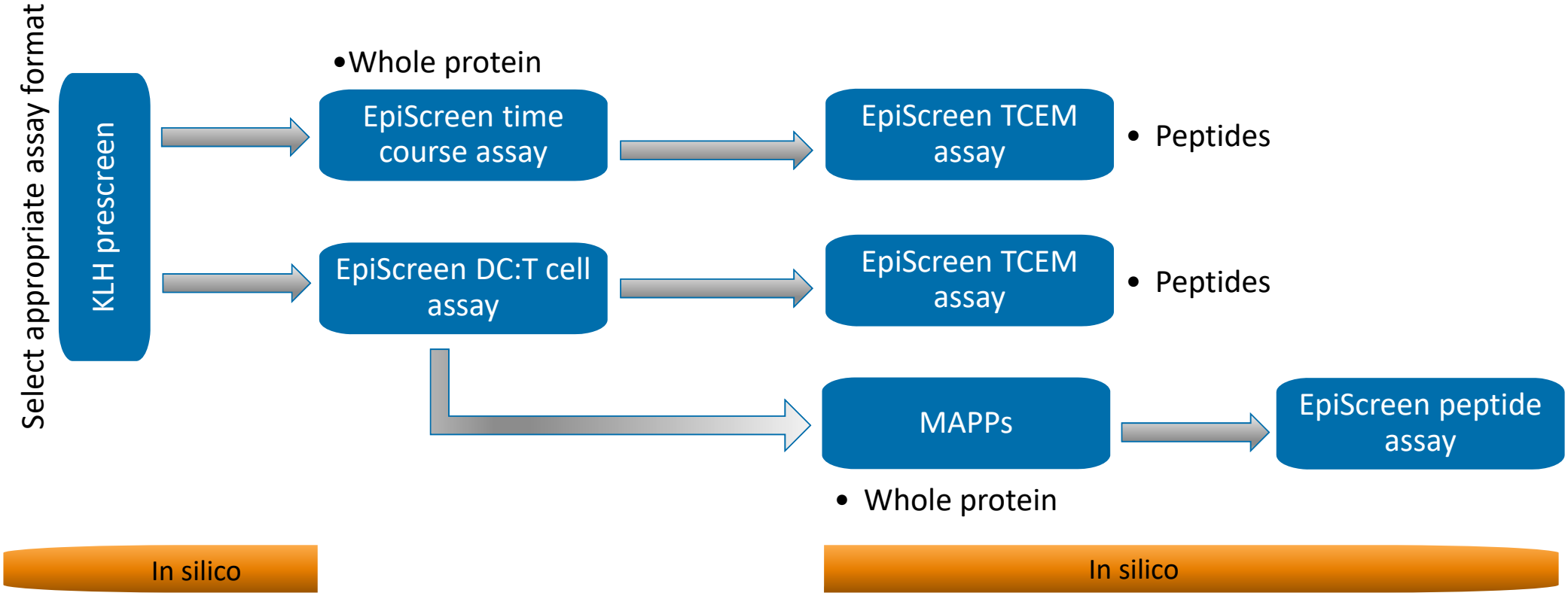


Donor Characterization and Quality Control

- High Resolution HLA-DR/DQ haplotyping
- Standard peptide/antigens:
  - Recall antigens= *CEFT, PPD*
  - Neoantigen = *KLH*
- Controlled cryopreservation process

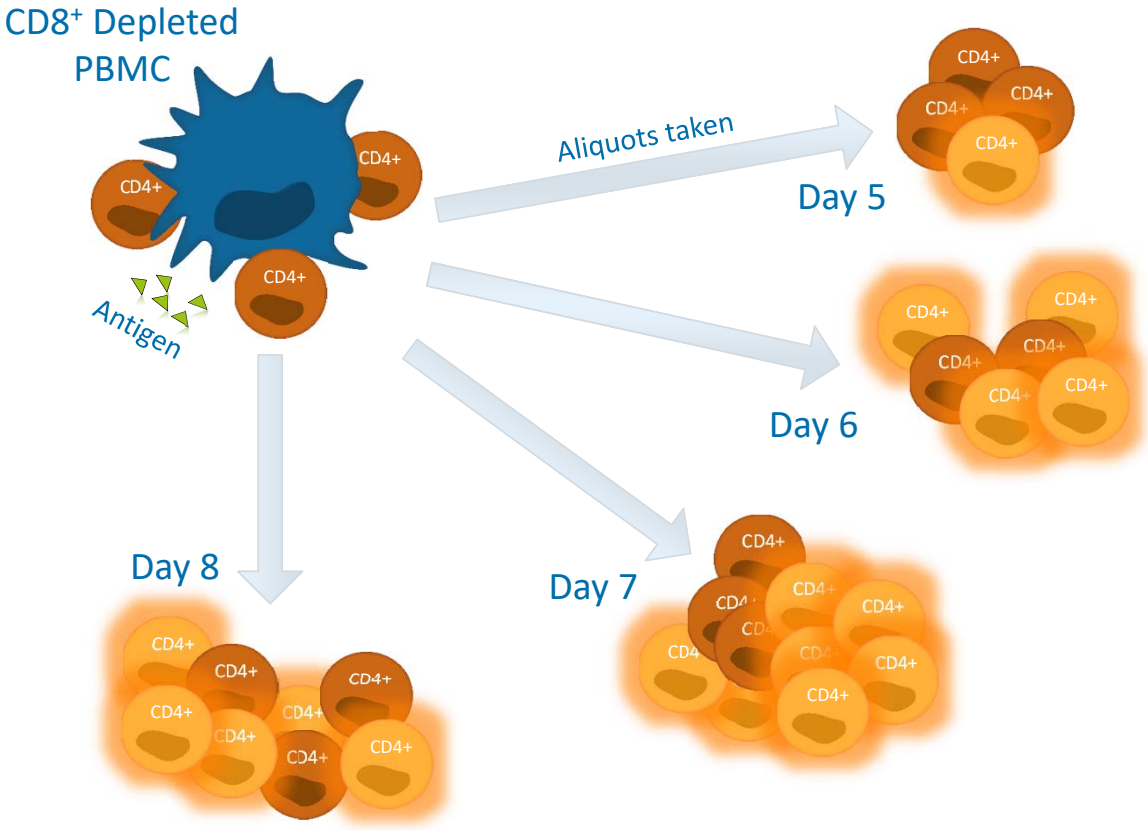
ABZENA
Enabling better biopharmaceuticals

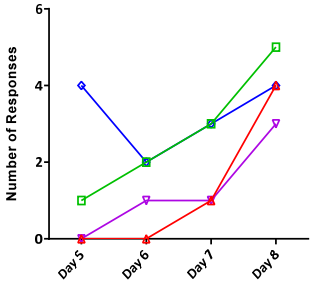
Appropriate Assay Format



ABZENA
Enabling better biopharmaceuticals

EpiScreen™ T cell Time Course Assay

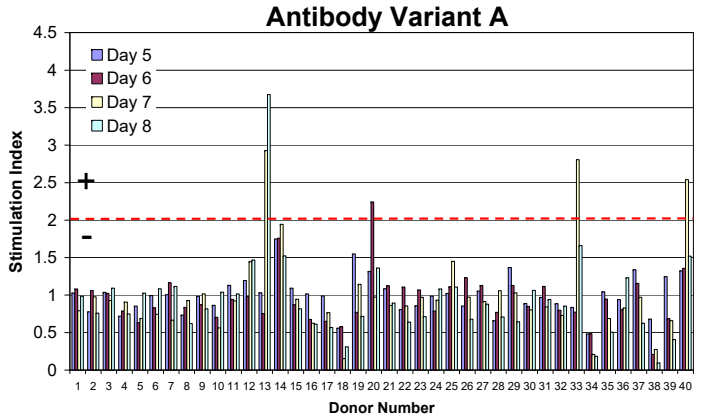


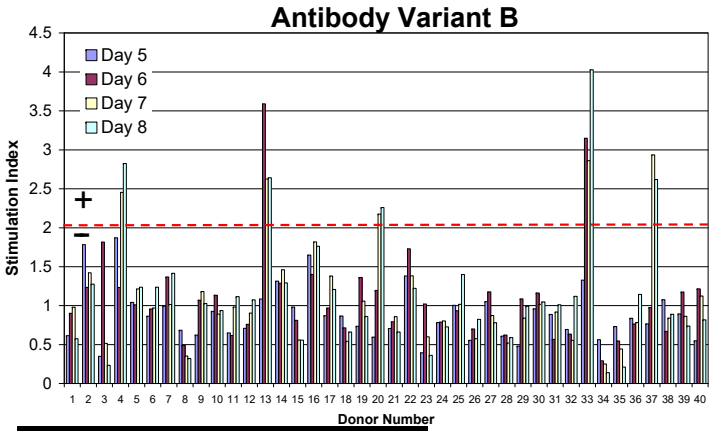


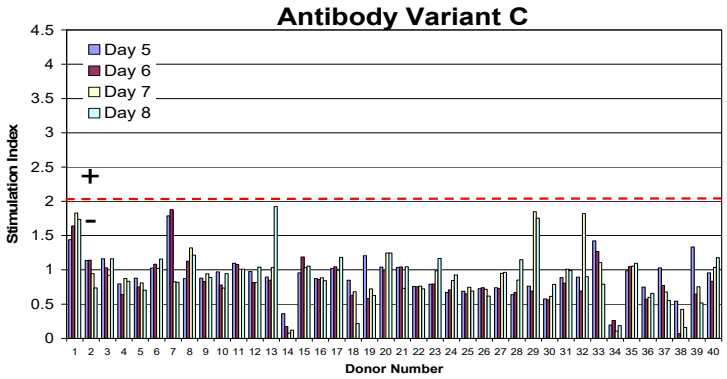
- Not suitable for biologics that can modulate KLH-specific T cell responses

ABZENA
Enabling better biopharmaceuticals

Lead selection: Humanised antibodies







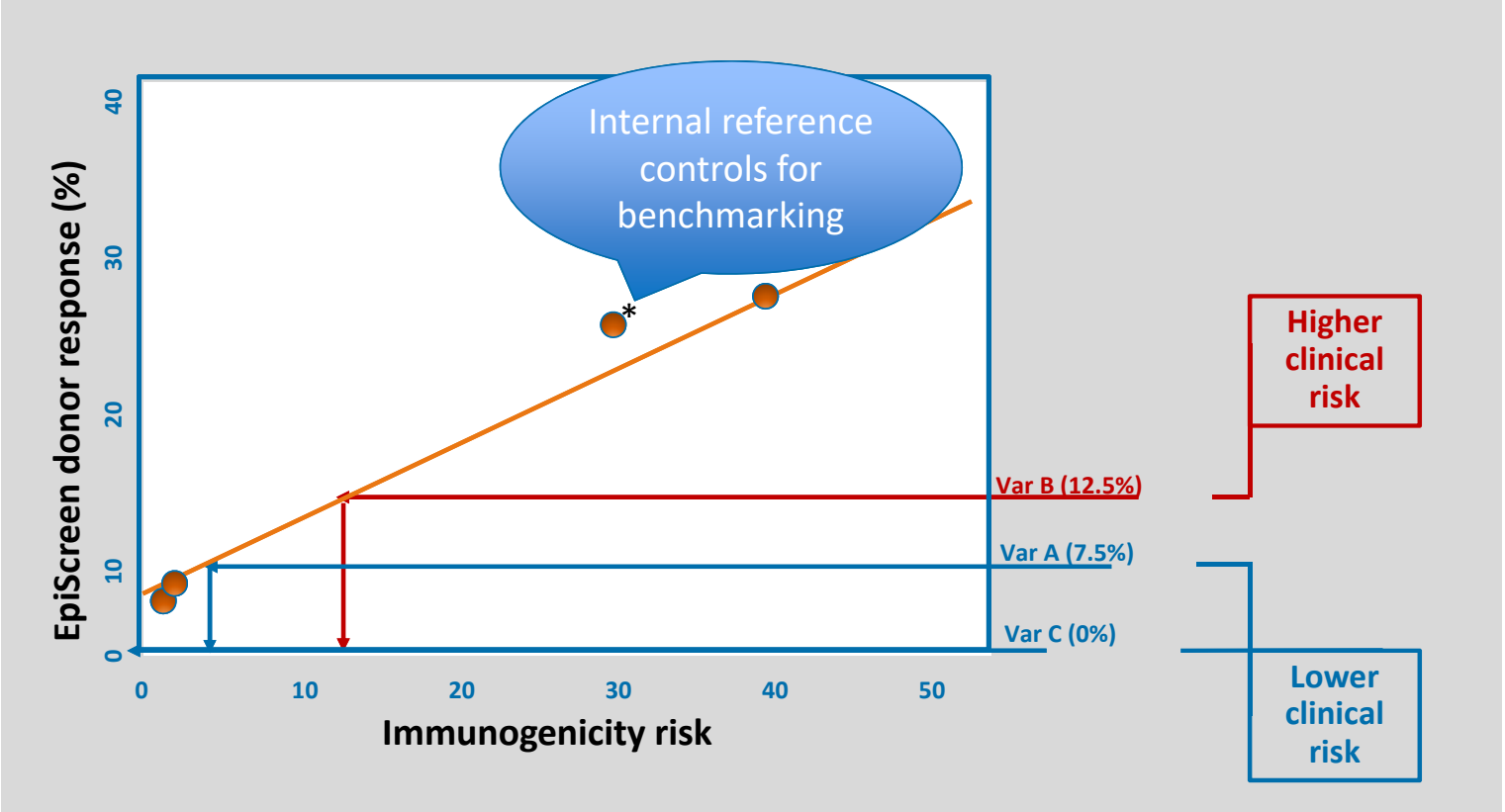
Donor #	Variant A	B	C
4		+	
13	+	+	
20	+	+	
33	+	+	
37		+	
40	+		
% Frequency	7.5	12.5	0
% Correlation	75	100	100
Mean SI	2.71	3.3	0

Positive IL-2 Elispot Assay

\+ Positive Proliferation Assay

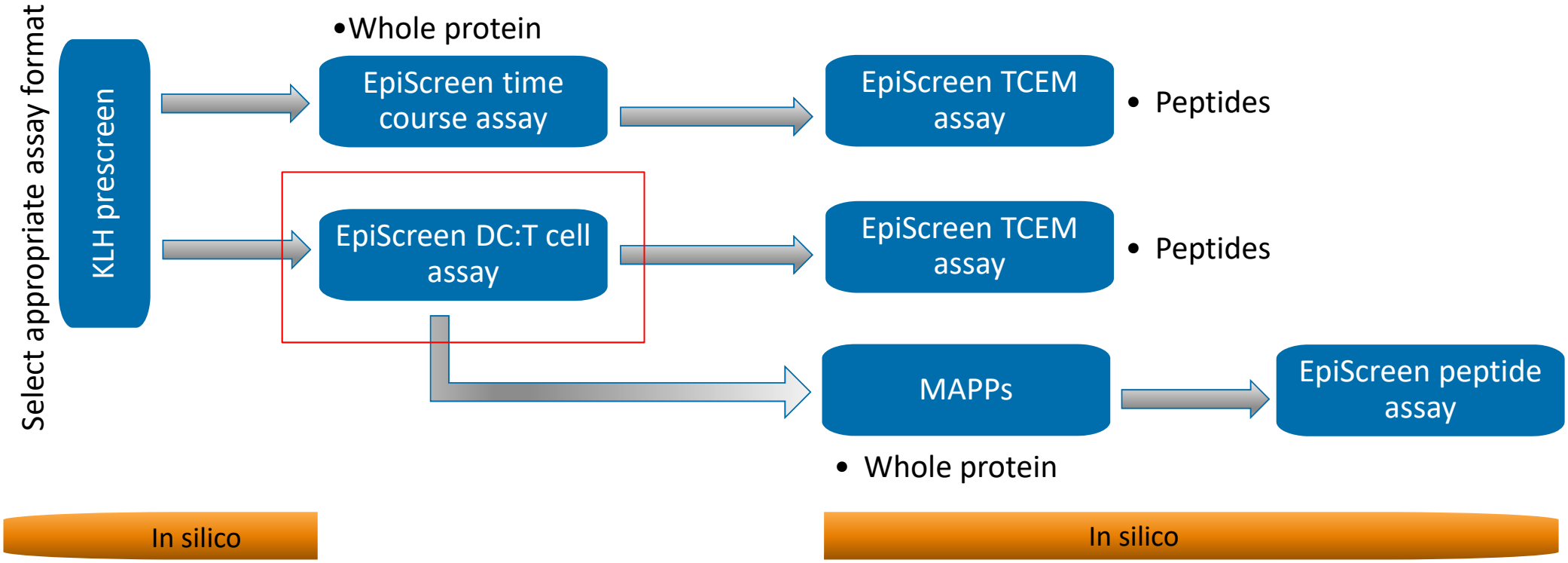
ABZENA
Enabling better biopharmaceuticals

EpiScreen™ Data Informs of Relative Risk of Test Product Candidates



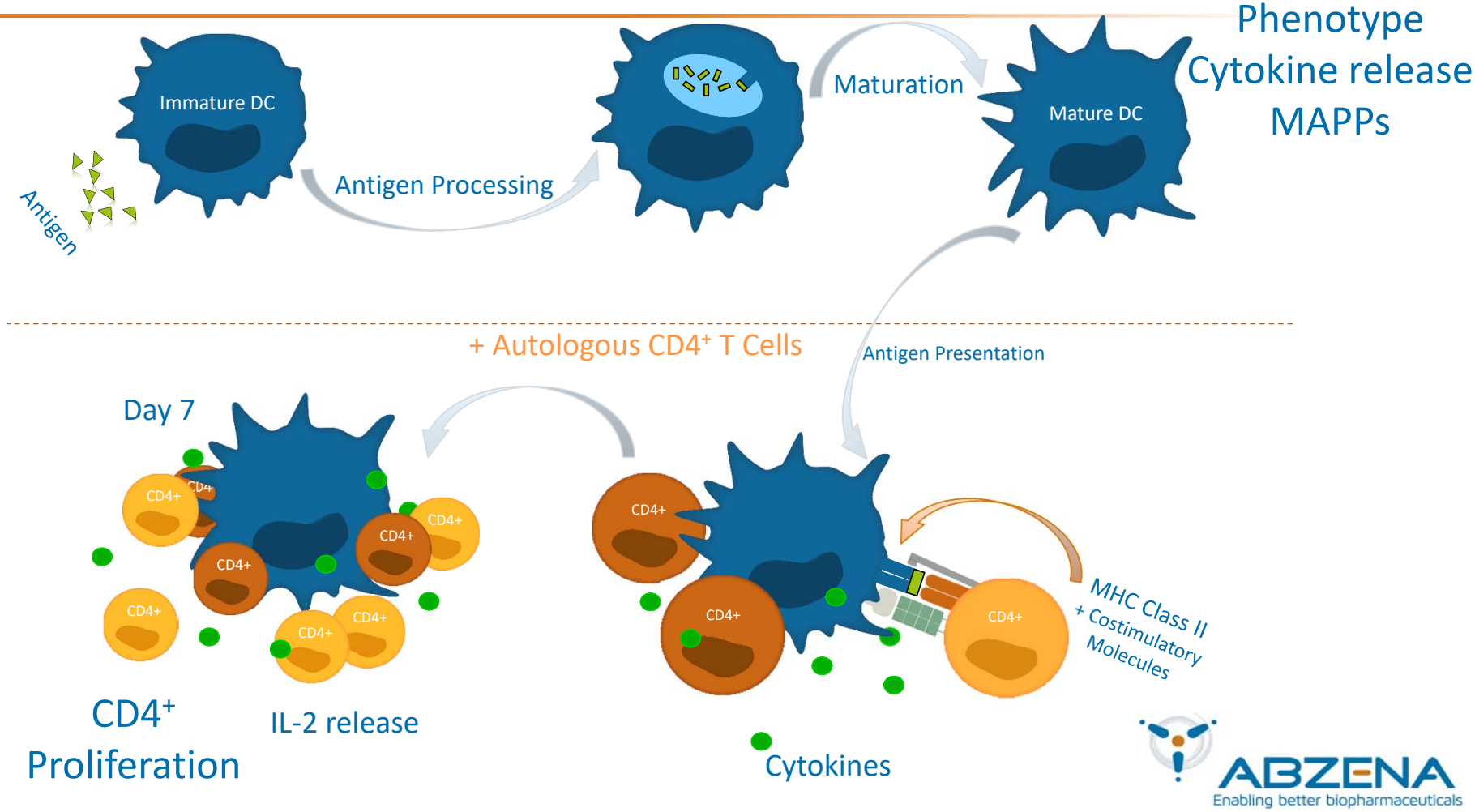
ABZENA
Enabling better biopharmaceuticals

Appropriate Assay Format



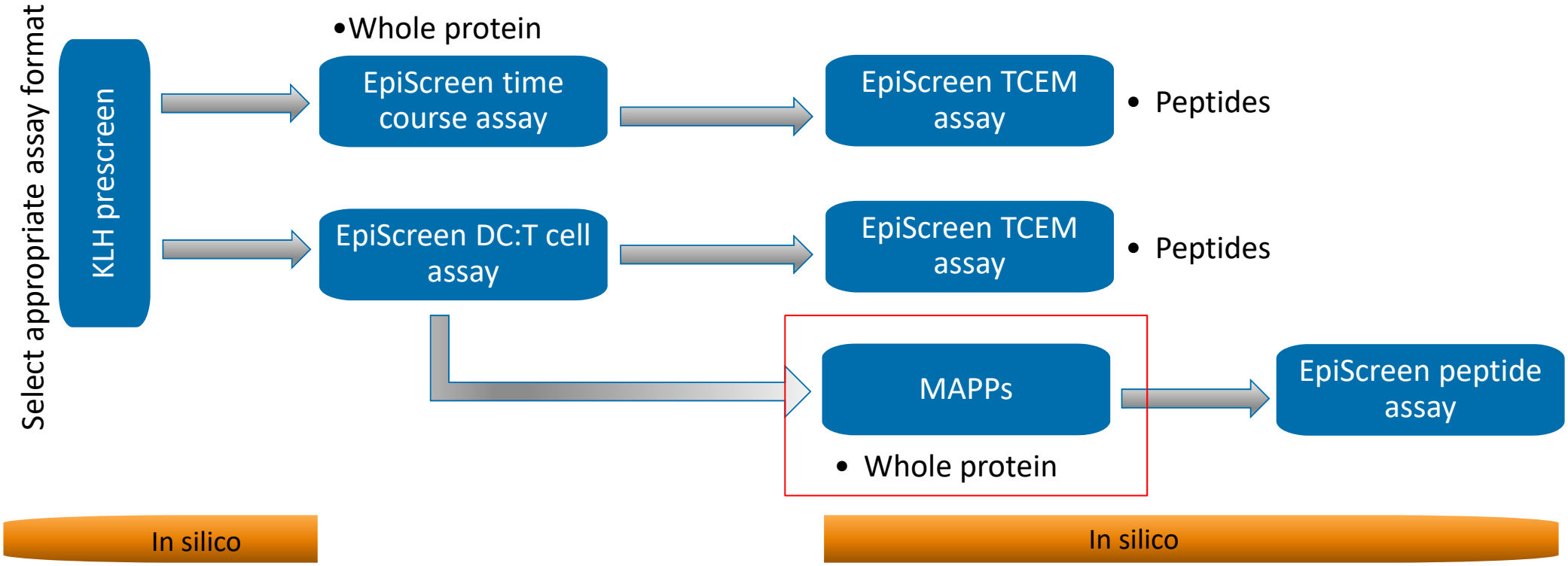
ABZENA
Enabling better biopharmaceuticals

EpiScreen™ DC:T cell Assay



ABZENA
Enabling better biopharmaceuticals

Appropriate Assay Format



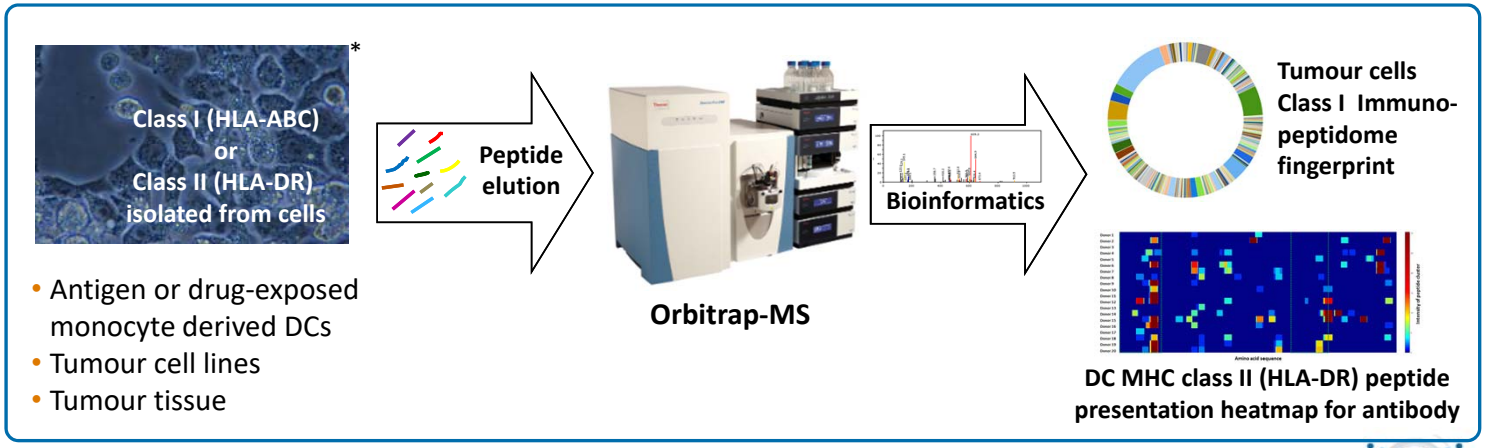
ABZENA
Enabling better biopharmaceuticals

MHC-Associated Peptide Proteomics (MAPPs)

MAPPs is a highly sensitive means of detecting and sequencing naturally processed and presented peptides (putative T cell epitopes) in the context of both MHC Class I and Class II.

Process Outline

- Class I and Class II molecules are isolated from cells (in vitro or ex vivo).
- MHC-bound peptides are eluted and run through a high powered Mass Spec to establish a profile of peptide sequences derived from extracellular or intracellular proteins.

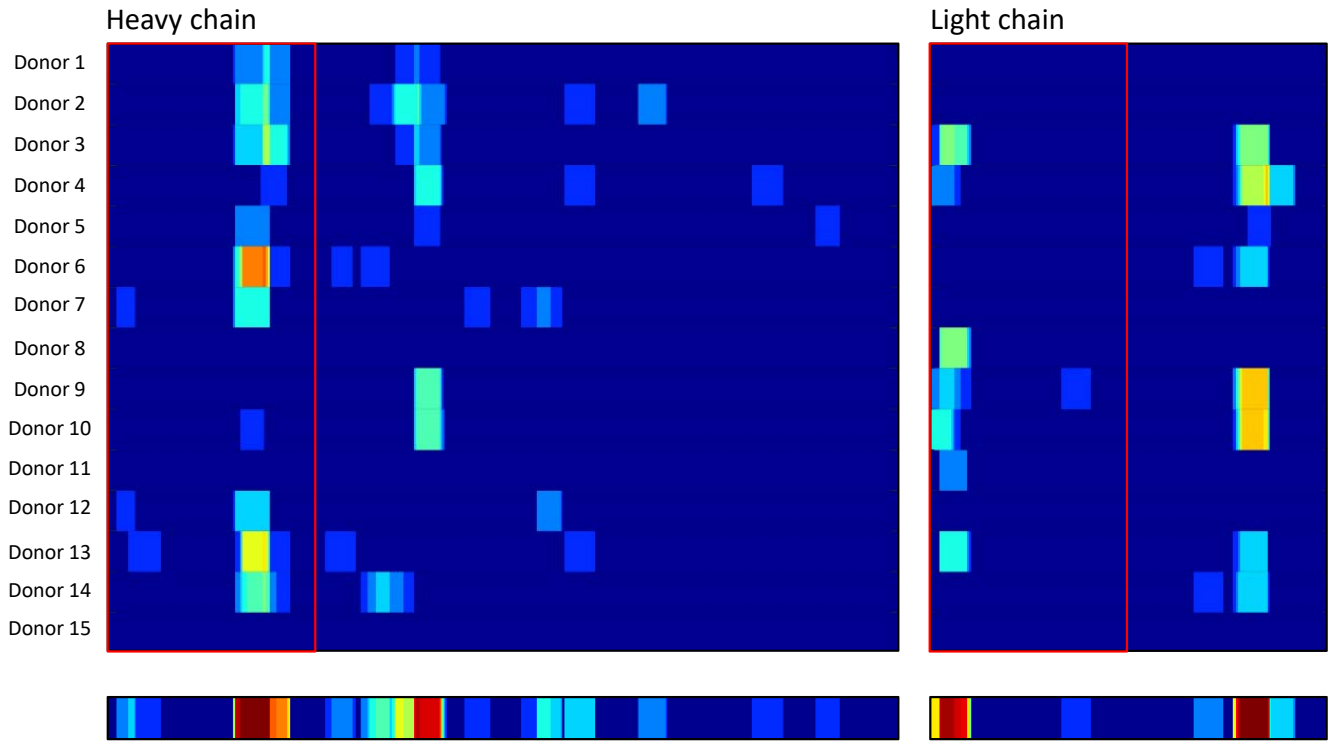


- Antigen or drug-exposed monocyte derived DCs
- Tumour cell lines
- Tumour tissue

*Image derived from: https://en.wikipedia.org/wiki/Hep_G2

ABZENA
Enabling better biopharmaceuticals

MAPPs – Infliximab Peptide Clusters



- Clusters identified in individual donors
- Clusters identified common to multiple donors

ABZENA
Enabling better biopharmaceuticals

MAPPs Sequence of Clusters in Infliximab

a.a. location of clusters

Cluster frequency within cohort

No	Location	Cluster sequence	Position	Frequency within cohort of donors (%)
1	31-46	RYGMSWVRQTPDKRLE	VH	6.7
2	40-58	TPDKRLELVAMMKTKGGRTY	VH	26.7
3	54-68	GGRTYYPDSVKGRFT	VH	13.3
4	62-77	SVKGRFTISRDNAKNS	VH	6.7
5	69-88	ISRDNAKNSLYLQMSSLKSEDTA	VH	66.7
6	134-151	GTAALGCLVKDYFPEPVT	CH	6.7
7	159-174	LTSGVHTFPAVLQSSG	CH	6.7
8	172-188	SSG…PSSS	CH	33.3
9	184-204	VPS…KPSN	CH	40

Sequence of clusters

	Donor No													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0	0	0	0	0	0	0	14.3	0	0	0	0	0	0
0	0	0	0	12.5	0	0	20.8	0	0	0	100	6.7	0	0
0	0	0	0	0	0	0	0	0	2.6	0	0	0	0	5.6
0	0	0	0	0	0	0	0	0	0	12.5	0	0	0	0
0	0	39.5	0	12.5	87.5	100	58.3	0	25.6	62.5	0	26.7	66.7	44.4
0	0	0	0	0	0	0	0	0	5.1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	7.7	0	0	0	0	0
0	0	15.8	17.4	0	0	0	0	4.8	0	6.3	0	0	0	5.6
75	0	0	13	25	0	0	0	0	0	0	0	6.7	4.2	5.6

Peptide abundance by individual donor

ABZENA
Enabling better biopharmaceuticals

Summary

- Immunogenicity continues to be a concern for the development of biotherapeutics from a safety, efficacy and cost perspective
- Many factors can contribute to immunogenicity but CD4+ T cell epitopes are the principal drivers of immunogenicity *in vivo*
- Multiple in vitro assays are available using human cells

Immunogenicity risk evaluation of aggregates

- Overview of select published data
- Utilising human cell in in vitro assays to evaluate risk of aggregated biologics

Aggregated EPO induced T cell responses

T-cell assays confirm immunogenicity of tungsten-induced erythropoietin aggregates associated with pure red cell aplasia

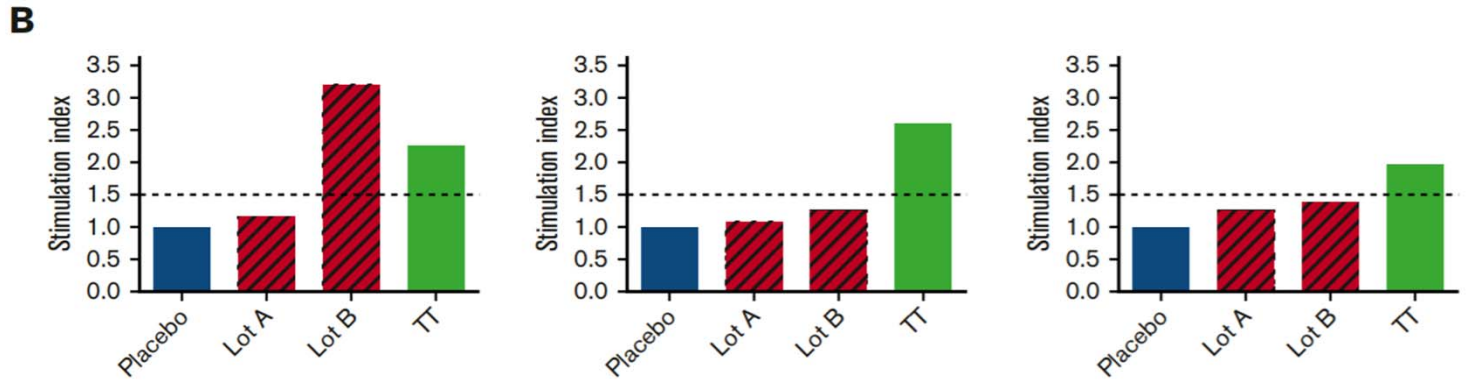
Tina Rubic-Schneider,[1] Masataka Kuwana,[2] Brigitte Christen,[1] Manuela Aßenmacher,[3] Otmar Hainzl,[3] Frank Zimmermann,[3] Robert Fischer,[3] Vera Koppenburg,[4] Salah-Dine Chibout,[1] Timothy M. Wright,[5] Andreas Seidl,[3] and Michael Kammüller[1]

[1]Discovery and Investigative Safety, Novartis Institutes for Biomedical Research, Basel, Switzerland; [2]Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan; [3]Technical Development Biosimilars, Novartis Biologics Technical Development and Manufacturing, Oberhaching, Germany; and [4]Sandoz Biopharmaceuticals, Oberhaching, Germany; and [5]Novartis Pharma Development, Basel, Switzerland

- Lot of EPO HX575 associated with PRCA
- Authors able to obtain blood samples from PRCA patient
- Also HX575 used to induce immune responses in healthy donors to EPO

ABZENA
Enabling better biopharmaceuticals

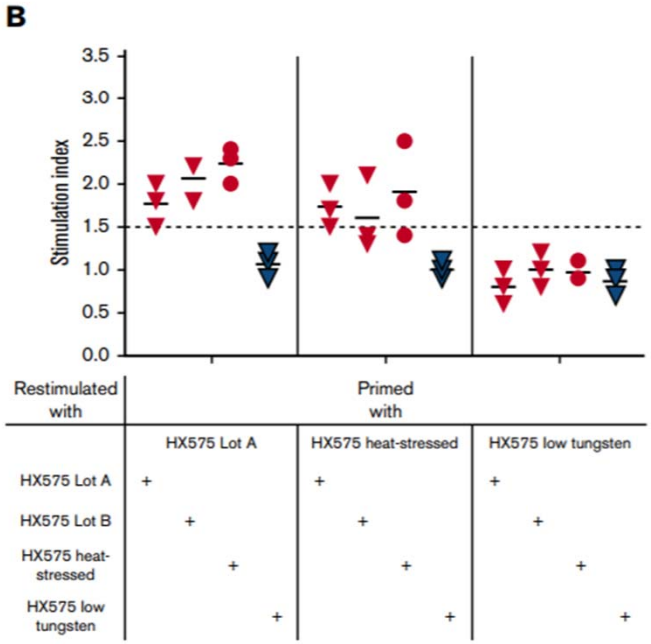
Aggregated EPO induced T cell responses

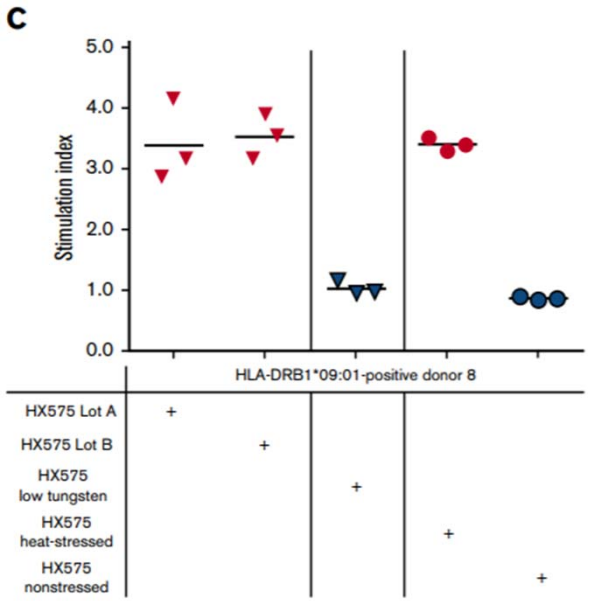


- All patients responded to tetanus toxin recall antigen
- Patient 1 had rhEPO neutralising antibodies after treatment with HX575 lot B
- Patient 1 had recall response to lot B

ABZENA
Enabling better biopharmaceuticals

Aggregated EPO induced T cell responses





- Healthy donors stimulated with HX575 lot A or heat stressed HX575 resulted in EPO specific T cell responses

ABZENA
Enabling better biopharmaceuticals

Aggregated EPO induced T cell responses

- Tungsten containing clinical lots and heat-stressed research lots were able to induce T cell responses in vitro
- Non-stressed showed no impact on T cell responses
- PRCA patient sample showed EPO-specific T cell responses in vitro

Highly Aggregated Antibody Therapeutics Can Enhance the *in Vitro* Innate and Late-stage T-cell Immune Responses[S]

Received for publication, December 5, 2011, and in revised form, May 8, 2012 Published, JBC Papers in Press, May 14, 2012, DOI 10.1074/jbc.M111.330902

Marisa K. Joubert[‡1], Martha Hokom[§], Catherine Eakin[¶], Lei Zhou[‖], Meghana Deshpande[§], Matthew P. Baker[**], Theresa J. Goletz[‡‡], Bruce A. Kerwin[§§], Naren Chirmule[§], Linda O. Narhi[‡], and Vibha Jawa[§2]

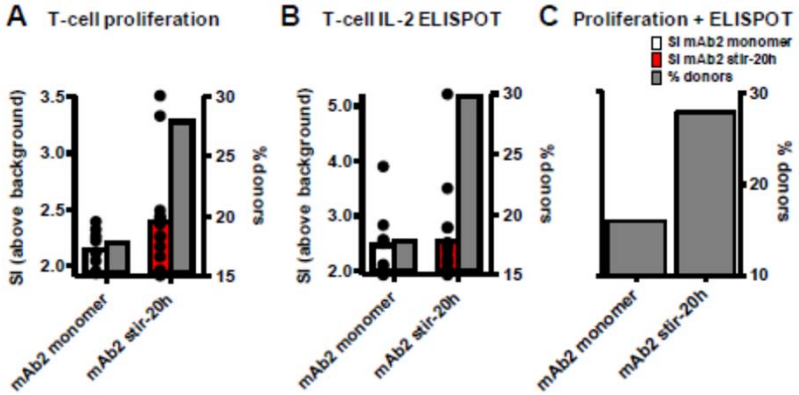
From the Departments of [‡]Product Attribute Sciences, [§]Clinical Immunology, and [‖]Medical Sciences, Amgen Inc., Thousand Oaks, California 91320, the Departments of [¶]Drug Substance Development, [§§]Drug Product Development, and [‡‡]Clinical Immunology, Amgen Inc., Seattle, Washington 98119, and [**]Antitope Ltd., Babraham Institute, Cambridge CB22 3AT, United Kingdom

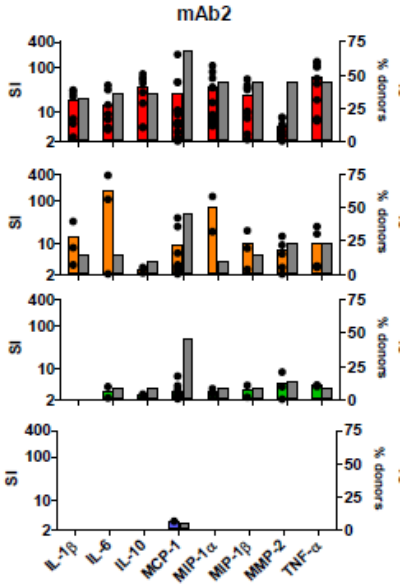
Background: Aggregated biotherapeutics have the potential to induce an immune response.
Results: Aggregates can enhance innate and adaptive immune responses of PBMC.
Conclusion: The response depends on aggregate type, immunogenicity of the monomer, donor immune status, and high particle numbers in the *in vitro* assay.
Significance: This is the first study showing the impact of aggregate characteristics on the potential immune response of PBMC.

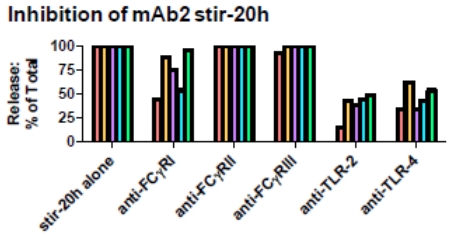
ABZENA
Enabling better biopharmaceuticals

Immune activation in response to stress-induced aggregates

- Stir stress >500,000 micron size particles/ml
- Activation of T cells
  - Proliferation
  - IL-2 production
- Stimulation of innate cytokines
- Different forms of stress resulted in different profiles
- Different antibodies gave different responses
- Responses could be inhibited







ABZENA
Enabling better biopharmaceuticals

Aggregation of a Wide Array of Clinical Biologics

RESEARCH ARTICLE

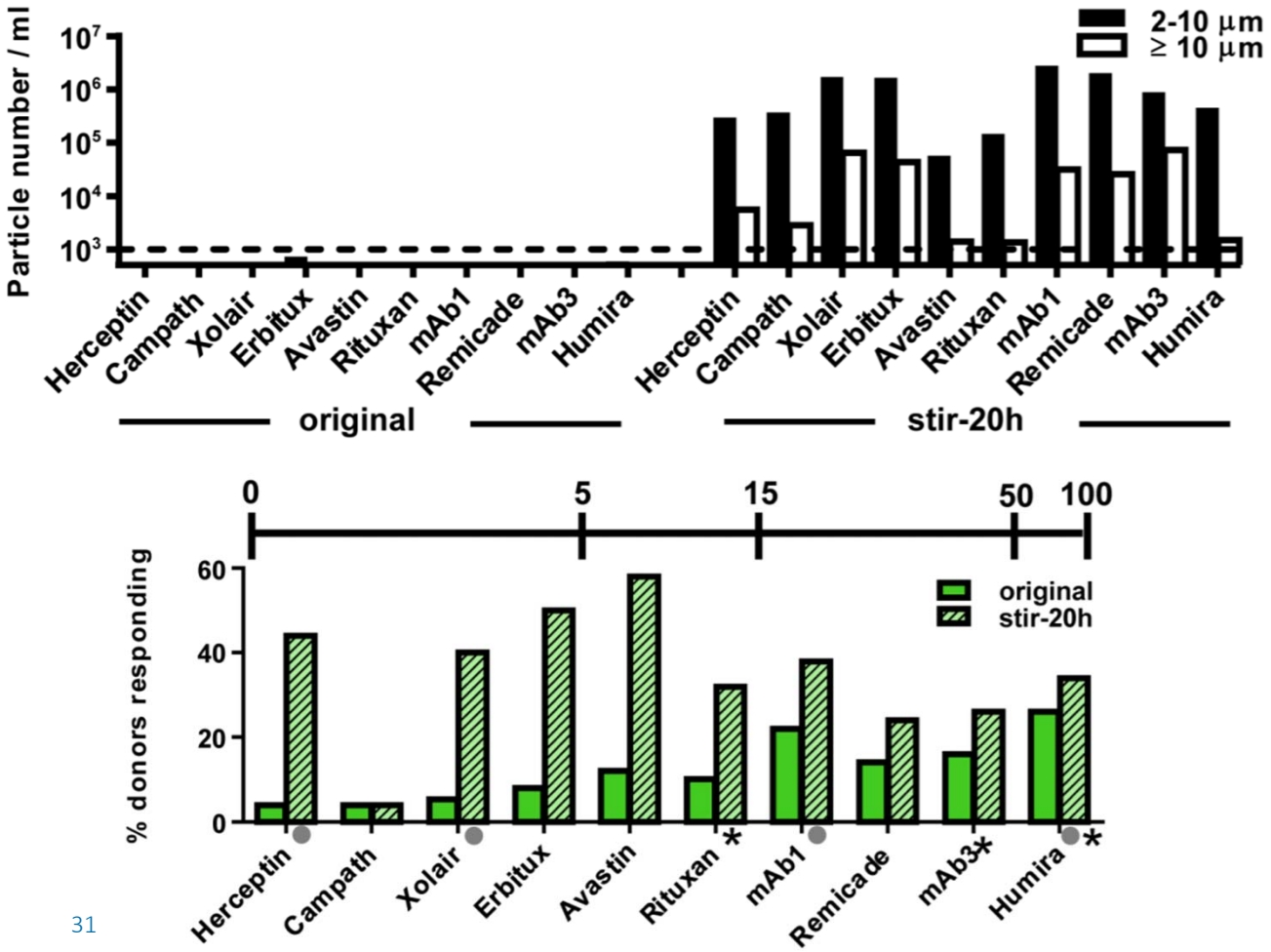
Use of *In Vitro* Assays to Assess Immunogenicity Risk of Antibody-Based Biotherapeutics

Marisa K. Joubert[1]*, Meghana Deshpande[2¤a], Jane Yang[1], Helen Reynolds[3], Christine Bryson[3¤b], Mark Fogg[3], Matthew P. Baker[3], Jonathan Herskovitz[2], Theresa J. Goletz[4], Lei Zhou[5], Michael Moxness[2], Gregory C. Flynn[1], Linda O. Narhi[1], Vibha Jawa[2]*

1 Department of Attribute Sciences, Amgen Inc., Thousand Oaks, California, United States of America, **2** Department of Clinical Immunology, Amgen Inc., Thousand Oaks, California, United States of America, **3** Antitope Limited, Babraham Research Campus, Cambridge, United Kingdom, **4** Department of Clinical Immunology, Amgen Inc., Seattle, Washington, United States of America, **5** Department of Medical Sciences, Amgen Inc., Thousand Oaks, California, United States of America

¤a Current address: Eurofins Lancaster Laboratories PSS, Thousand Oaks, California, United States of America
¤b Current address: Department of Haematology, University of Cambridge, Cambridge, United Kingdom
* mjoubert@amgen.com (MJ); vjawa01@amgen.com (VJ)

ABZENA
Enabling better biopharmaceuticals



- Stir stress induced significant aggregation
- Biologics with low risk of clinical immunogenicity have increased risk when aggregated

ABZENA
Enabling better biopharmaceuticals

Immune activation in response to stress-induced aggregates

Pharm Res
DOI 10.1007/s11095-014-1541-x

RESEARCH PAPER

Small Amounts of Sub-Visible Aggregates Enhance the Immunogenic Potential of Monoclonal Antibody Therapeutics

Maryam Ahmadi • Christine J. Bryson • Edward A. Cloake • Katie Welch • Vasco Filipe • Stefan Romeijn • Andrea Hawe • Wim Jiskoot • Matthew P. Baker • Mark H. Fogg

- Comparative analysis of rituximab and trastuzumab stress induced aggregate particles
- Used less stressed material

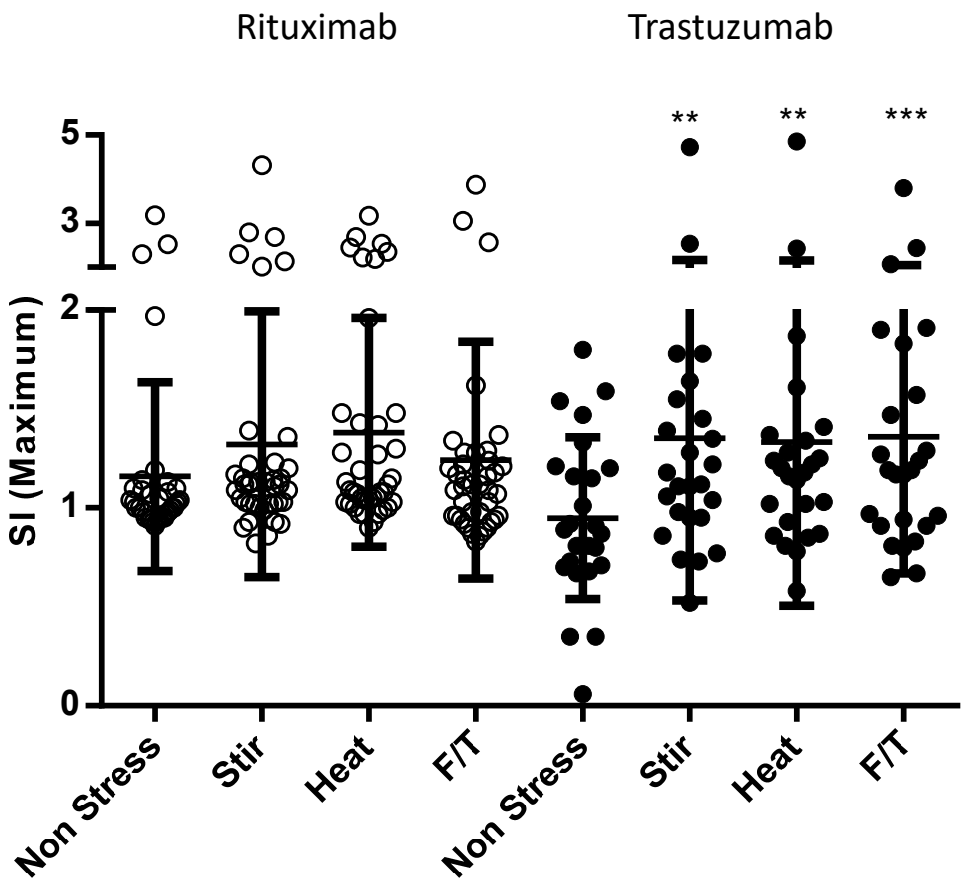
ABZENA
Enabling better biopharmaceuticals

Immune activation in response to stress-induced aggregates

- Overall stress-induced aggregates comprise <3% of total protein
- ~90% less >1um size particles in rituximab samples under stir stress conditions
- More representative of clinical products
- As observed previously mechanical stress induced highest levels of micron sized particles

Antibody	Stress condition	HP-SEC		DLS		Light obscuration (particles/ml)		
		Recovery, relative to unstressed (%)	Monomer content (%)	Z-average diameter (nm)	PDI	>1 um	>10 um	>25 um
Rituximab	Unstressed	100	99.1	10.2 ± 0.3	0.09 ± 0.02	287	58	0
	Stir (200rpm/30min)	103.1	99.1	1540 ± 134	1.00 ± 0.00	2,125	10	0
	Heat (70°C/10min)	98.7	99.3	19.7 ± 0.4	0.38 ± 0.14	1,314	29	19
	Freeze/thaw 10 cycles	100.5	98.9	10.1 ± 0.1	0.18 ± 0.01	520	19	0
Trastuzumab	Unstressed	100	97.5	10.3 ± 0.1	0.14 ± 0.02	904	0	10
	Stir (200rpm/30min)	96.7	97.7	2203 ± 857	0.77 ± 0.24	33,073	67	0
	Heat (70°C/10min)	96.9	97.6	28.7 ± 6.2	0.11 ± 0.01	1,021	10	0
	Freeze/thaw 10 cycles	95.6	96.8	53.3 ± 30.1	0.14 ± 0.03	10,404	67	0

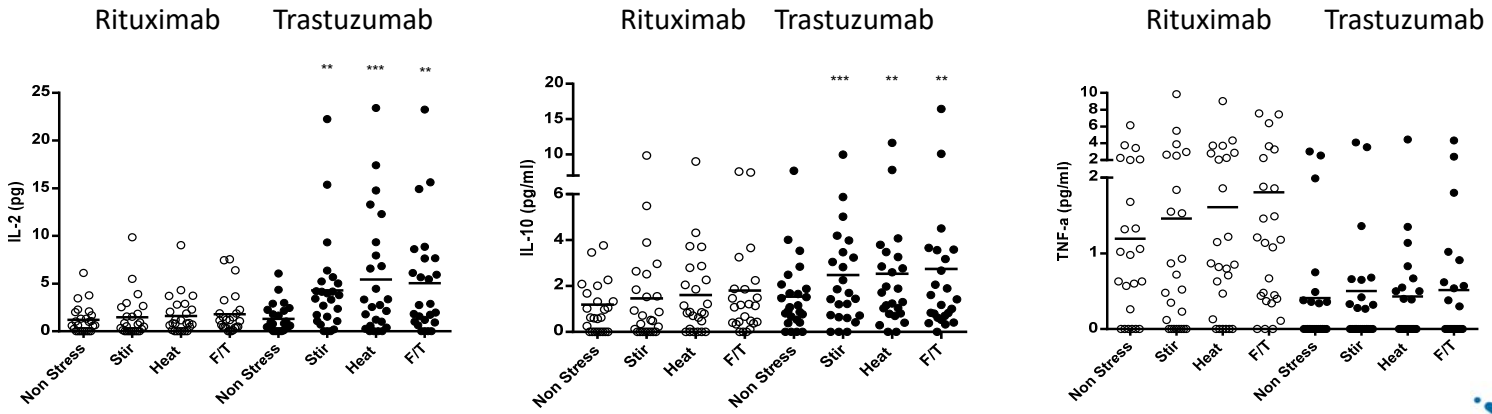
Effects of aggregates on proliferation of PBMC



ABZENA
Enabling better biopharmaceuticals

Effect of aggregates on cytokine production by PBMC

- Increased IL-2 and IL-10 production associated with increased proliferation to trastuzumab
- Monomeric rituximab increased TNF-α compared to trastuzumab
- Variable levels of other proinflammatory cytokines
- mDC phenotyping revealed increased expression of activation markers e.g. CD83, CD86 and HLA

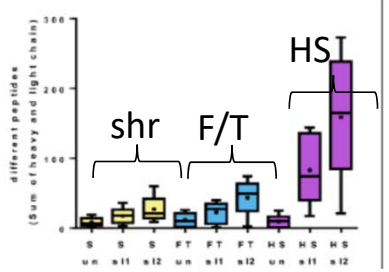


ABZENA
Enabling better biopharmaceuticals

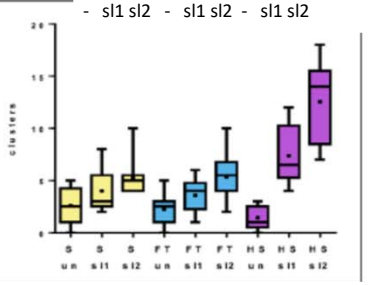
Immune Activation in Response to Stress-Induced Aggregates

Compound	aa chain		Stress condition and level: S un	S sl1	S sl2		FT un	FT sl1	FT sl2		HS un	HS sl1	HS sl2
mAb 1	different peptides (Σ heavy and light chain)	Donor S 1	2	7	9	Donor FT 1	19	35	62	Donor HS 1	0	33	81
		Donor S 2	8	9	21	Donor FT 2	2	2	5	Donor HS 2	14	141	273
		Donor S 3	10	30	39	Donor FT 3	10	22	31	Donor HS 3	7	80	203
		Donor S 4	4	8	14	Donor FT 4	26	34	54	Donor HS 4	10	68	165
		Donor S 5	13	18	19	Donor FT 5	1	5	22	Donor HS 5	10	61	122
		Donor S 6	17	36	60	Donor FT 6	14	34	64	Donor HS 6	25	120	217
		Donor S 7	19	21	22	Donor FT 7	10	18	45	Donor HS 7	19	144	260
		Donor S 8	0	3	14	Donor FT 8	22	33	62	Donor HS 8	0	N/A	21
		Donor S 9	4	N/A	N/A	Donor FT 9	0	1	2	Donor HS 9	1	17	88
		Donor S 10	6	23	45	Donor FT 10	10	37	74				
						Donor FT 11	1	9	35				
						Donor FT 12	23	40	65				
		Average	8.3	17.2	27.0	Average	11.5	22.5	43.4	Average	9.6	83.0	158.9
		SEM	1.9	3.6	5.4	SEM	2.6	4.1	6.7	SEM	2.7	15.8	27.2
	clusters (Σ heavy and light chain)	Donor S 1	2	4	4	Donor FT 1	3	3	4	Donor HS 1	0	4	7
		Donor S 2	3	2	4	Donor FT 2	2	2	4	Donor HS 2	3	11	16
		Donor S 3	4	8	10	Donor FT 3	3	5	4	Donor HS 3	1	6	14
		Donor S 4	2	3	4	Donor FT 4	1	2	2	Donor HS 4	1	7	14
		Donor S 5	5	5	5	Donor FT 5	1	3	6	Donor HS 5	2	5	10
		Donor S 6	3	3	4	Donor FT 6	4	6	10	Donor HS 6	2	8	12
		Donor S 7	5	6	6	Donor FT 7	3	4	8	Donor HS 7	3	12	18
		Donor S 8	0	2	5	Donor FT 8	5	4	6	Donor HS 8	0	N/A	7
		Donor S 9	1	N/A	N/A	Donor FT 9	0	1	2	Donor HS 9	1	6	15
		Donor S 10	1	3	5	Donor FT 10	1	5	7				
						Donor FT 11	1	4	6				
						Donor FT 12	3	4	5				
		Average	2.6	4.0	5.2	Average	2.3	3.6	5.3	Average	1.4	7.4	12.6
		SEM	0.5	0.6	0.6	SEM	0.4	0.4	0.6	SEM	0.4	0.9	1.2

different peptides and clusters per stress condition and level



- sl1 sl2 - sl1 sl2 - sl1 sl2



Legend													
color coding peptides													
number of different peptides	0	1	10	20	30	40	50	60					
color coding clusters													
number of clusters		1	2	3	4	5	6	7	8	9	10	11	≥ 12

Rombach-Riegraf et al. 2014; PLoS ONE 9(1): e86322. doi:10.1371/journal.pone.0086322

ABZENA better biopharmaceuticals

Summary

- Regardless of the immunogenicity risk of monomeric clinical therapeutics, the presence of small quantities (<3%) of aggregates can induce proliferation of PBMC
  - T cell proliferation and cytokine production
- Low levels of particles/aggregates achieve this by activating the innate immune response
  - Detected by DC activation and cytokine secretion
  - Leads to changes in peptides presented to the immune response
- Clinically relevant material but not prepared under ‘clinical use conditions’

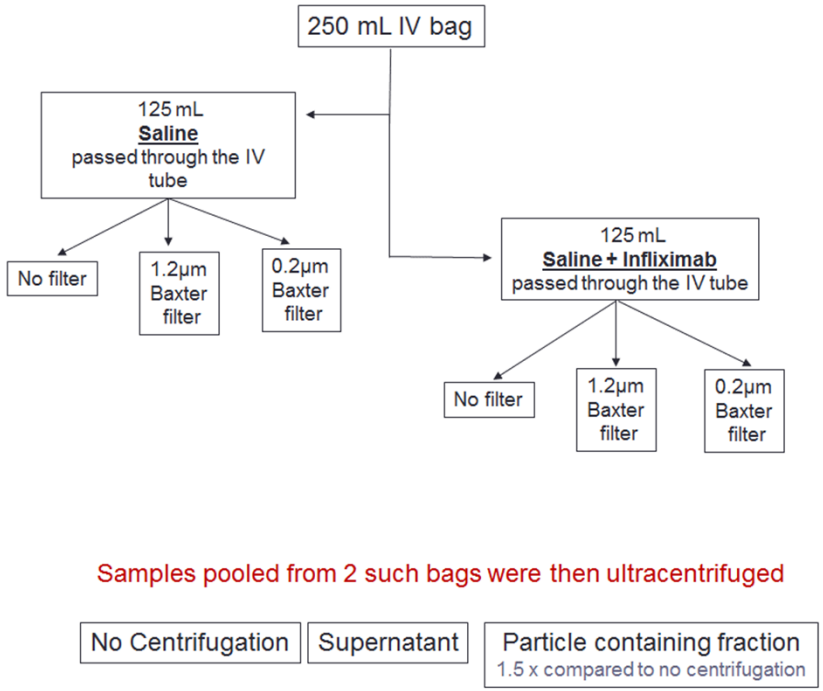
Immune responses to clinically dispensed therapeutic proteins

- Due to administration via IV infusion, in addition to immunogenic responses; infusion reactions are also observed in patients

- Infliximab: More than 40% of the patients turn non-responders
  - 10-25% of the patients have hypersensitivity/infusion reactions*

- Hypothesis: Particles in IV solution of Infliximab may play a role in eliciting infusion related reactions and immunogenic responses

Sample Preparation and in vitro Assay

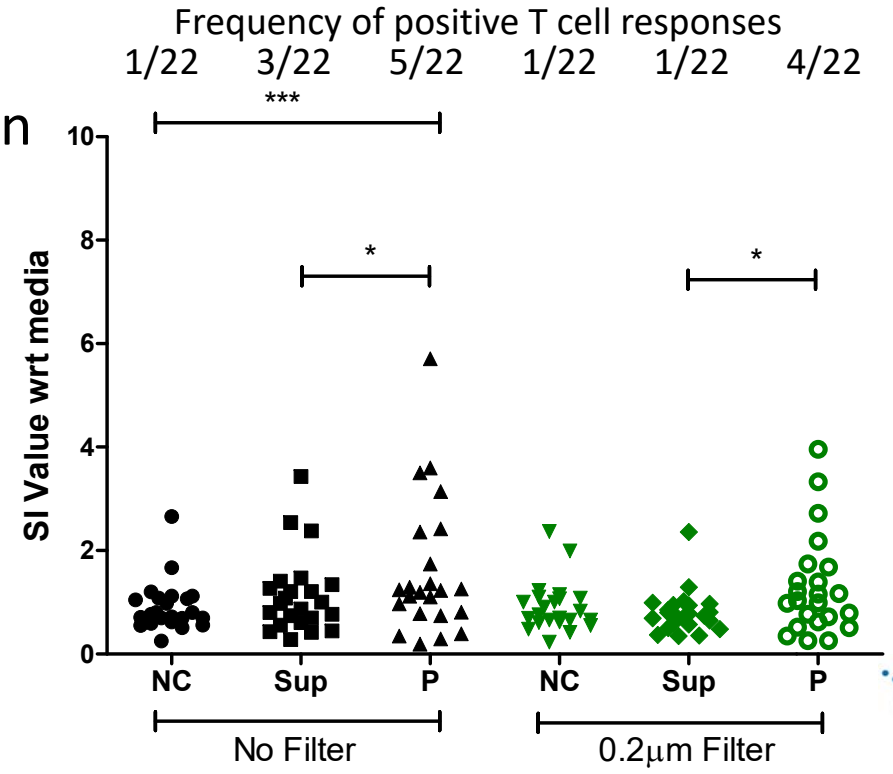


- T cell proliferation assay

ABZENA
Enabling better biopharmaceuticals

Stimulation of T cell proliferation by particles

- Particle enriched fraction had a higher response compared to no centrifugation sample
- Both pre and post filtration



ABZENA
Enabling better biopharmaceuticals

Summary

- Infusion reaction is regulated by the number of particles present in solution:
  - No filter > Filtered samples and
  - Particle containing fraction > No centrifugation
- T cell proliferation may also be impacted by the nanoparticle content:
  - Particle containing fraction > No centrifugation
- IV solution of Infliximab led to activation of the innate and the adaptive immune response

Conclusions

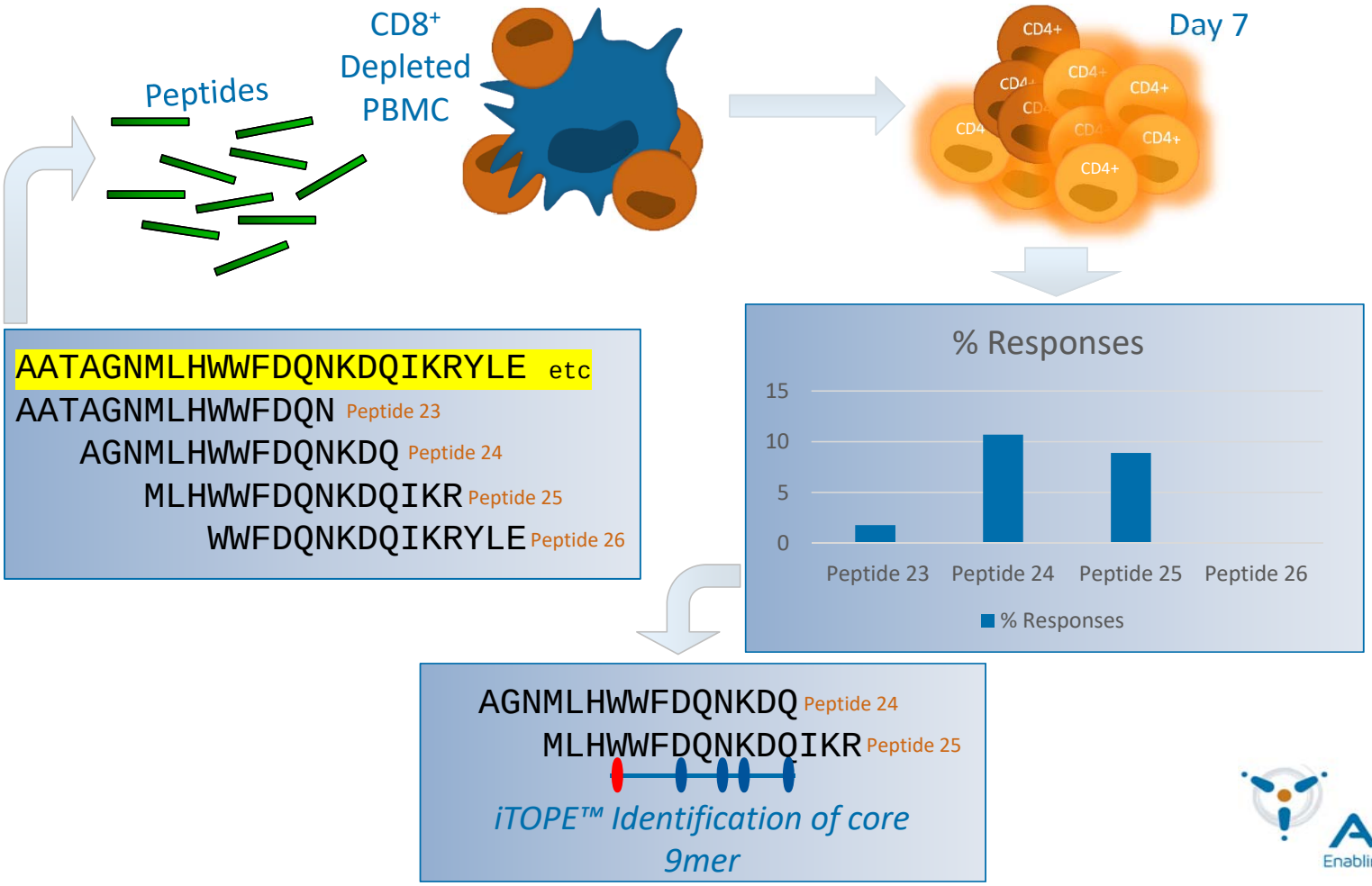
- Using human cells in in vitro assays allows
  - Determination of the relative risk of biologics stimulating ADA in vivo
  - Determination of the ability of aggregates to activate the innate immune response
  - Determination of the ability of aggregates to activate the adaptive immune response
- Can be used not only for risk evaluation of new biologics
- Impurities
- Reformulation
- Change in delivery

Acknowledgements

- Immunology group
  - Maryam Ahmadi
  - Helen Reynolds
  - Edward Cloake
  - Alyson Rust
- Bioanalytical group
  - Ricardo Resende
- University of Colorado
  - John Carpenter
  - Neha Pardeshi
- Amgen
  - Marisa Joubert
  - Vibha Jawa

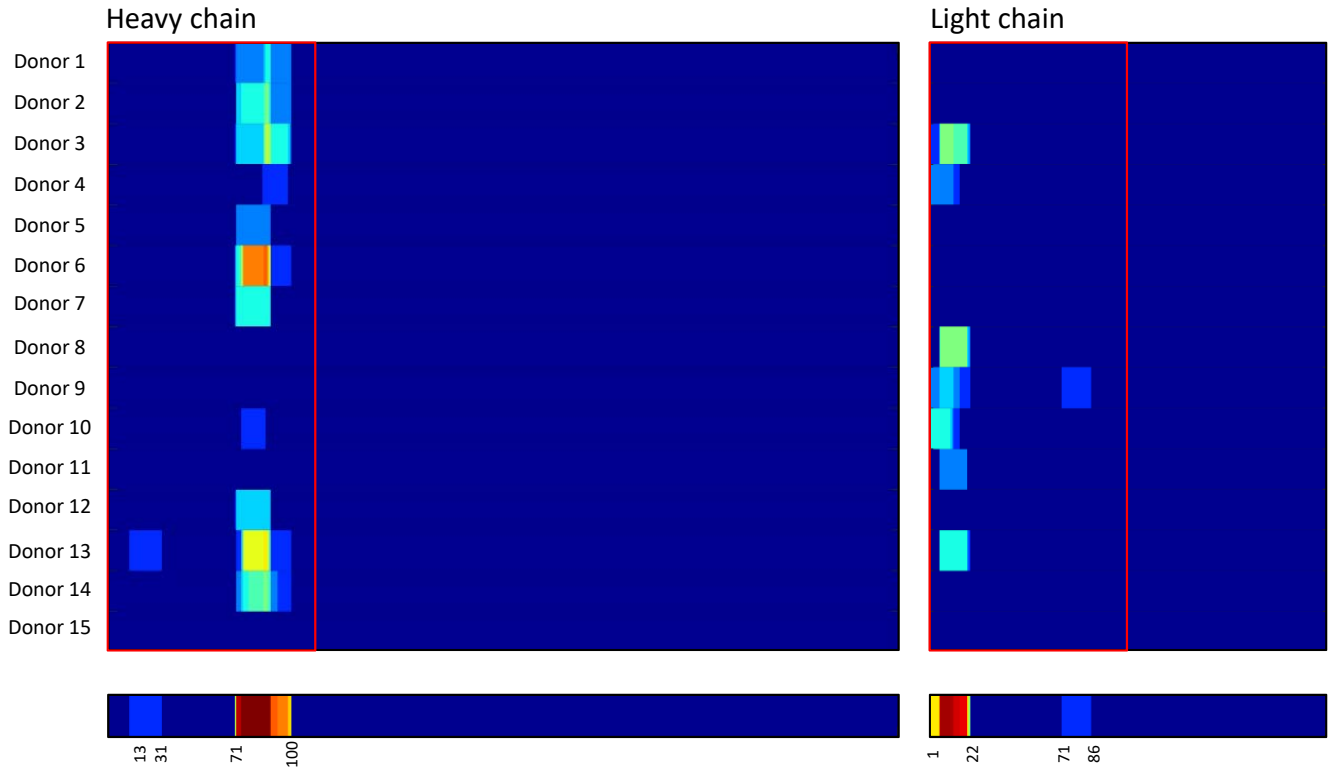
ABZENA
Enabling better biopharmaceuticals

EpiScreen™ T cell Epitope Mapping (TCEM)



ABZENA
Enabling better biopharmaceuticals

MAPPs Germline Filtering of Infliximab



- Clusters remain in the murine variable regions of the sequence

ABZENA
Enabling better biopharmaceuticals