Evolution of *in silico* tools for Predicting And in vitro assays for Validating Immunogenicity Risk

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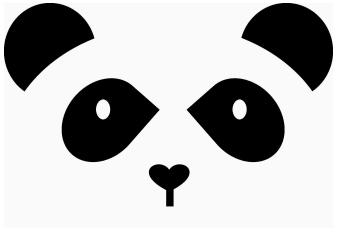
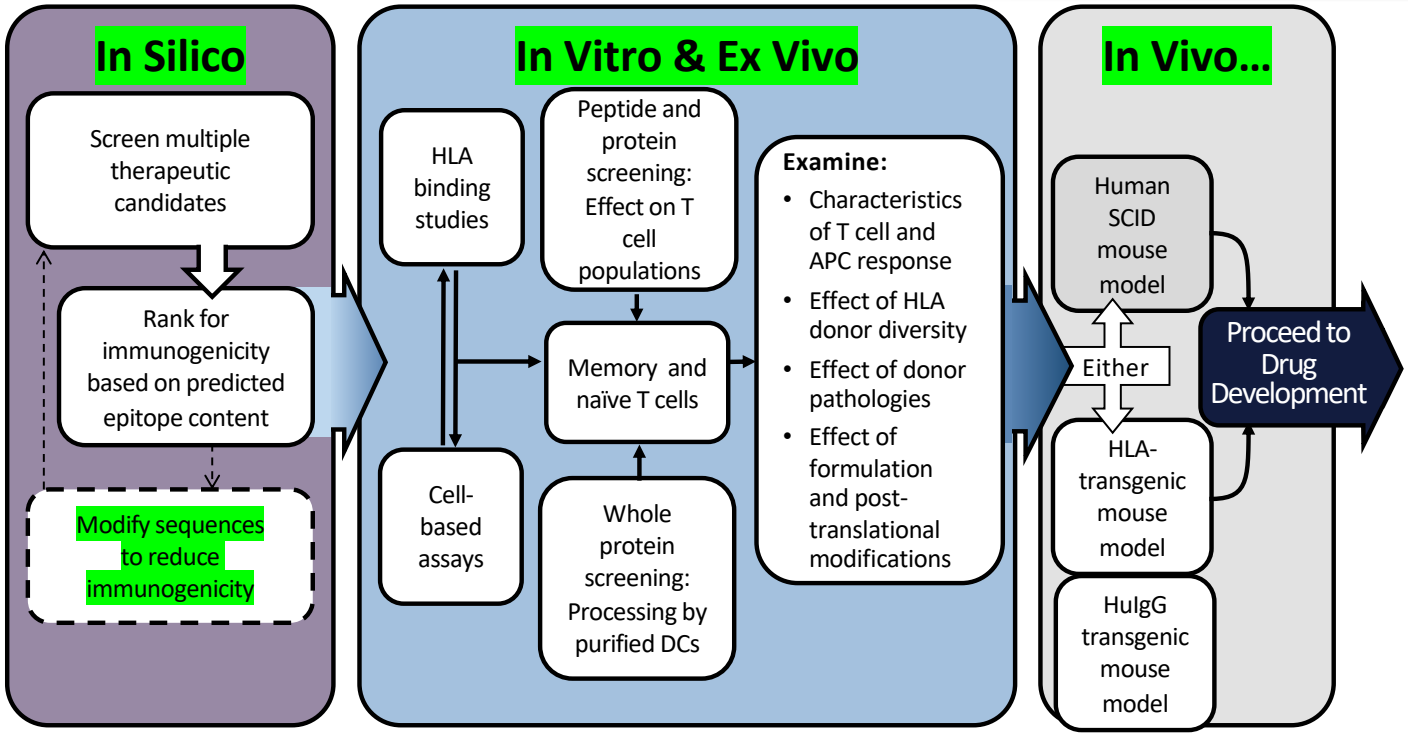


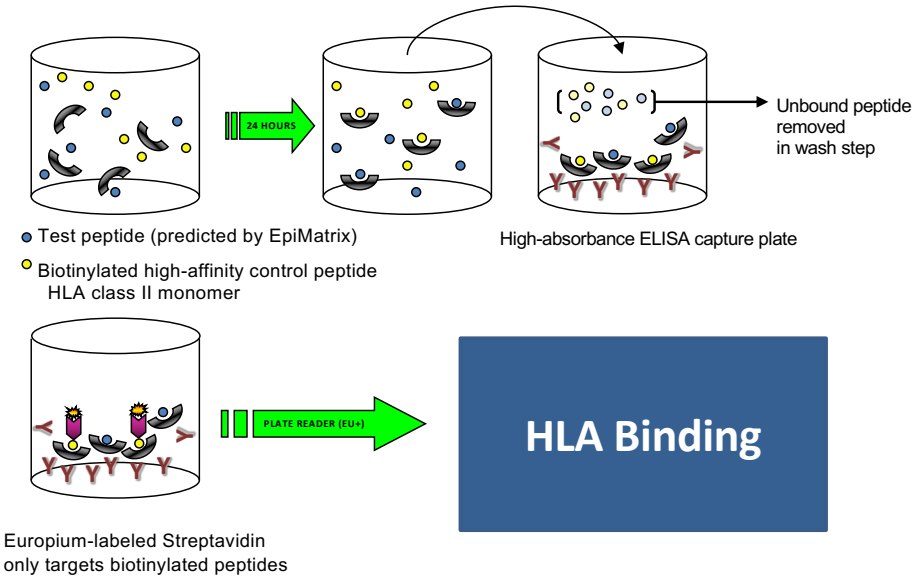
Immunogenicity Risk Assessement

EpiVax



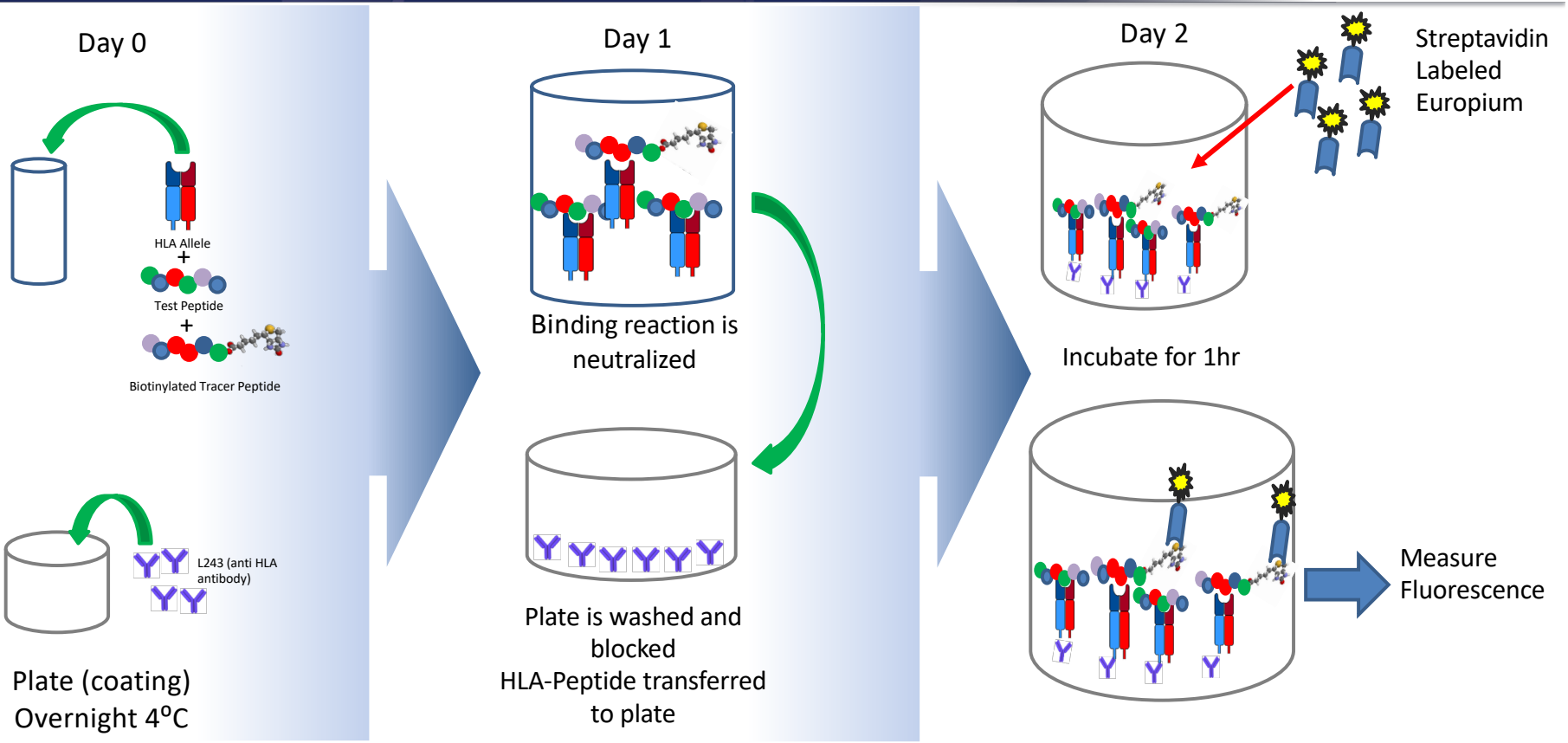
T-cell dependent immunogenicity of protein therapeutics: Preclinical assessment and mitigation.
Jawa V, Cousens LP, Awwad M, Wakshull E, Kropshofer H, **De Groot** AS. Clin Immunol. 2013 Dec;149(3):534-55. doi:10.1016/j.clim.2013.09.006. Epub 2013 Sep 25. Review. PMID: 24263283

HLA Binding Assay
Competition Assay Approach

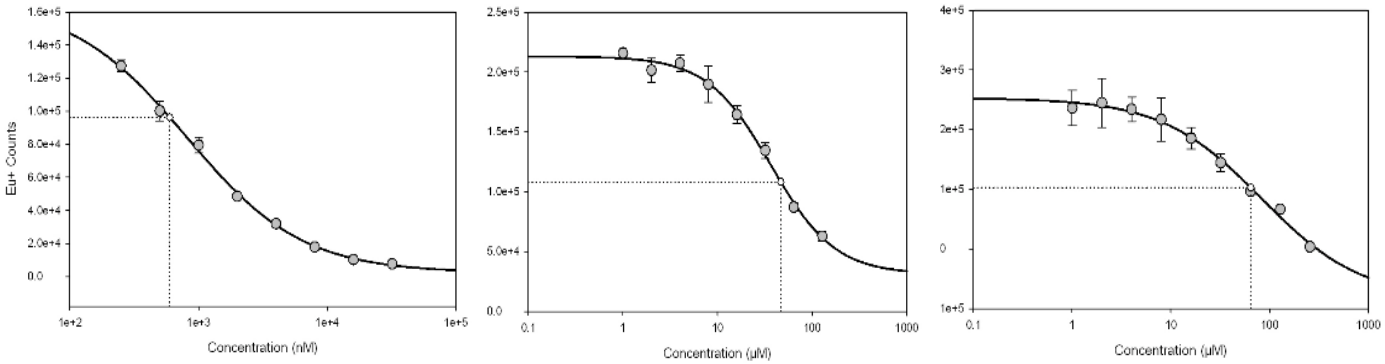


- Fluorescence counts are converted into percent inhibition of biotinylated standard
- Each test peptide is assayed at 8 concentrations ranging from 0 nm-100,000nm
- If dose-dependence curves are observed, IC_{50} values are calculated using GraphPad Prism software
- The lower the IC_{50} the higher the binding affinity of the test peptide

HLA-Binding Assay Overview



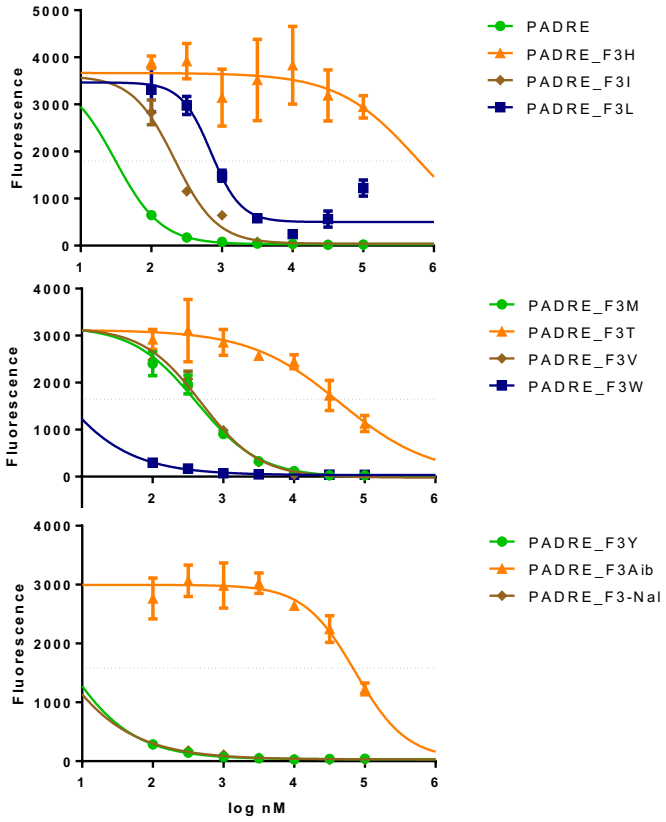
7 Point Binding Curve
EpiVax Sample Results



Strong binder | Moderate binder | Weak binder

The shape of each inhibition curve and the location of its intercept with 50% maximal binding dictates how a test peptide is characterized. EpiVax uses this information to delineate between strong, moderate and weak binders. Rating peptides by these criteria works in tandem with the detailed profile provided by displaying a representation of a peptide's entire effective range.

PADRE (substituting P1) HLA DRB1 0101 binding results



Peptide Sequence	HLA DRB1*0101	
	Max Epx	IC50
AK[1-Nal]VAAWTLKAAA	--	5.29
AK**W**VAAWTLKAAA	2.28	5.73
AK**Y**VAAWTLKAAA	2.24	6.73
AK**F**VAAWTLKAAA	2.27	31.27
AK**I**VAAWTLKAAA	1.90	218.74
AK**M**VAAWTLKAAA	1.60	388.09
AK**V**VAAWTLKAAA	1.71	447.45
AK**L**VAAWTLKAAA	1.84	789.92
AK**T**VAAWTLKAAA	0.94	38,975.54
AK[Aib]VAAWTLKAAA	--	68,080.25
AK**H**VAAWTLKAAA	1.11	N.B.
Correlation:		-0.820972956

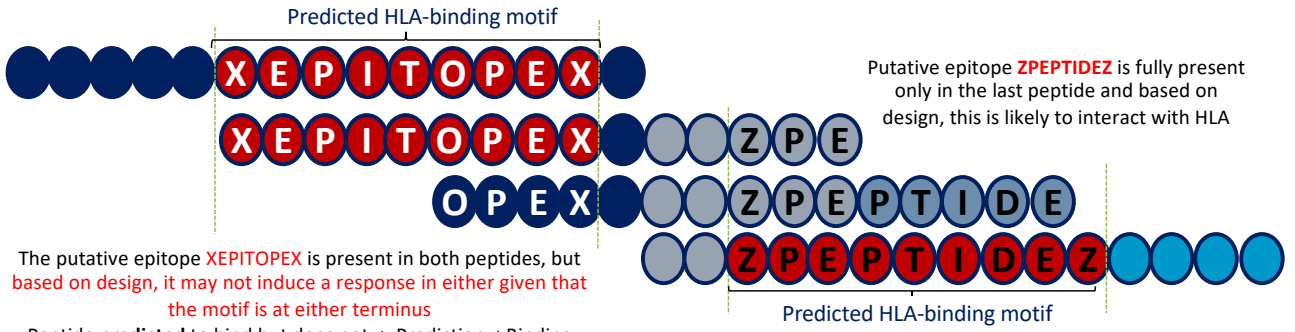
Max EPX = highest EpiMatrix score in peptide for given allele

IC50 = Concentration (nM) of tested peptide that competes 50% of maximum tracer peptide binding

IC50 (nM)
Non-binder
> 100,000 – Negligible Binder
10,000-100,000 – Weak Binder
1,000-10,000 – Moderate Binder
100-1,000 – Strong Binder
< 100 – Very Strong Binder

Peptide Flanking Residues are important

- **Flanking residues** at the ends of the core epitope, particularly the amino end, make contacts with the MHC molecule, **increasing stability of the pMHC complex**.
- **Poorly centered HLA-binding motifs** (at the N- or C- terminal of the binding peptide) may result in **absence of binding or T cell response.**



Putative epitope **ZPEPTIDEZ** is fully present only in the last peptide and based on design, this is likely to interact with HLA

The putative epitope XEPITOPEX is present in both peptides, but based on design, it may not induce a response in either given that the motif is at either terminus

Peptide **predicted** to bind but does not -> Prediction ≠ Binding

The truncated epitope (**ZPEPTIDE)** though *not predicted to bind***,** could also induce a response if residue at position 1 ("Z") is a strong P1 binding anchor,

Peptide **not predicted** to bind, but does -> Prediction ≠ Binding

HLA Binding Case Study from published literature (Hamze et al)

- We did a retrospective analysis of a recent publication that used **overlapping peptides to characterize T cell epitopes** from Infliximab and Rituximab.
- In silico predictions were compared to in vitro binding results: **results were discordant**.
- We hypothesized that the discordance could be due to **suboptimal peptide design:**
  - We tested in original and centered versions of a subset of these peptides in a Class II HLA binding assays to test this hypothesis

Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors

*Moustafa Hamze[1], Sylvain Meunier[1], Anette Karle[2], Abdelaziz Gdoura[1], Amélie Goudet[1], Natacha Szely[3], Marc Pallardy[3], Franck Carbonnel[4], Sebastian Spindeldreher[2], Xavier Mariette[5], Corinne Miceli-Richard[5] and Bernard Maillère[1]**

[1] CEA-Saclay, Institut de Biologie et Technologies, Université Paris-Saclay, Gif sur Yvette, France, [2] Novartis Pharma AG, Basel, Switzerland, [3] INSERM UMR 996, Faculté de Pharmacie, Université Paris-Sud, Chatenay Malabry, France, [4] Service de gastro-entérologie, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, France, [5] INSERM UMR 1184, Assistance Publique-Hôpitaux de Paris, Service de Rhumatologie, Hôpitaux Universitaires Paris-Sud, Université Paris-Sud, Le Kremlin-Bicêtre, France*

*The **chimeric antibodies anti-CD20 rituximab (Rtx) and anti-TNFα infliximab (Ifx)** induce antidrug antibodies (ADAs) in many patients with inflammatory diseases. Because of the key role of CD4 T lymphocytes in the initiation of antibody responses, we localized the CD4 T cell epitopes of Rtx and Ifx. With the perspective to anticipate immunogenicity of therapeutic antibodies, identification of the CD4 T cell epitopes was performed using cells collected in healthy donors. **Nine T cell epitopes were identified in the variable chains of both antibodies by deriving CD4 T cell lines raised against either Rtx or Ifx.** The T cell epitopes often exhibited a good affinity for human leukocyte antigen (HLA)-DR molecules and **were part of the peptides identified by MHC-associated peptide proteomics assay from HLA-DR molecules of dendritic cells (DCs)** loaded with the antibodies. Two-third of the T cell epitopes identified from the healthy donors stimulated peripheral blood mono-nuclear cells from patients having developed ADAs against Rtx or Ifx and promoted the secretion of a diversity of cytokines. These data emphasize the predictive value of evaluating the T cell repertoire of healthy donors and the composition of peptides bound to HLA-DR of DCs to anticipate and prevent immunogenicity of therapeutic antibodies.*

Sneak Preview of Findings

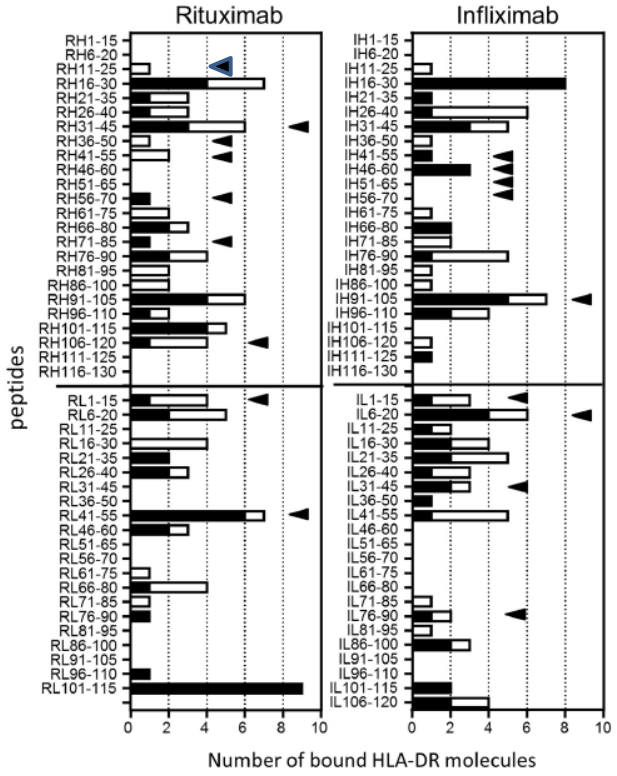
EpiVax

- We find that **centering binding motifs** in overlapping peptides yields **more binders** with **stronger affinities, improving association** of **in silico** predictions and **in vitro** findings**.**

- Careful attention should be taken to design peptides with optimal features, such as centered HLA binding motifs, before their usage in in vitro and in vivo experiments.

Hamze et al. EIP-funded 2017 RTX/IFX study

"In Silico tools don't predict Binding" according to authors



Do *in silico* predictions align with *in vitro* findings?

Methods: The observed binders in publication were compared to in silico predictions for the same (15 mer, overlapping) peptides, using EpiMatrix and IEDB consensus prediction methods.

Either: In Silico Tools do not Predict Binding

Or . . .

HLA Binding Assays As Performed Are Not Accurate

We set out to determine the Truth

Example: Malliere Non Binder → repeat assay

More sensitive assay confirms predictions

ORIGINAL

EpiMatrix Cluster Detail Report

20_IL1-15 Cluster: 1

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
1	DILLTQSPA	9	0.42	0.75	0.23	0.4	0.59	-0.02
2	ILLTQSPAI	10	1.31	2.27	1.65	2.45	1.24	2.64
3	LLTQSPAIL	11	1.23	2.07	1.51	1.32	1.76	1.35
4	LTQSPAILS	12	0.72	2	2.06	1.73	1.89	1.91
5	TQSPAILSV	13	0.77	-0.61	0.17	0.81	-0.27	0.15
6	QSPAILSVS	14	0.76	-0.66	-0.23	-0.83	-0.05	0.1
7	SPAILSVSP	15	0.97	-0.16	0.28	-0.11	-0.7	-0.22

Two strong EpiBars

Summarized Results	DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score	2.27	2.06	2.45	1.89	2.64
Publication Results (R.B.A)	B	NB	B	NB	B
EpiVax Assessment	B	B	B	B	TBD

Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors

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Observe binders in EpiVax HLA binding assay where publication did not

For Example - Where HLA Binding Results were negative

7 Point Curve and Centering Motif Correlations

EpiVax

ORIGINAL

EpiMatrix Cluster Detail Report

RH36-50 Cluster: 36

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
36	WVKQTPGRG	44	-1.3	2.26	1.93	1.24	2.31	1.22
37	VKQTPGRGL	45	-0.78	1.89	0.82	1.9	0.56	1.33
38	KQTPGRGLE	46	-1.63	-1.45	-1.83	-1	-0.9	-0.61
39	QTPGRGLEW	47	-1.3	-0.3	-0.29	0.22	-1.07	-0.45
40	TPGRGLEWI	48	-0.41	-1.98	-2.91	-1.66	-1.94	-1.72
41	PGRGLEWIG	49	-0.38	-1.19	-1.31	-1.56	-0.44	-0.59
42	GRGLEWIGA	50	0	-0.14	0.11	0.3	-0.04	0.34
Summarized Results				DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score				2.26	1.93	1.9	2.31	1.33
Publication Results				B	NB	NB	NB	NB

OPTIMIZED

EpiMatrix Cluster Detail Report

RH36-50MOD Cluster: 33

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
33	NMHWVKQTP	41	-0.27	-1.53	-0.52	-1.08	-0.07	-0.55
34	MHWVKQTPG	42	-0.19	1.07	0.48	0.26	0.36	0.71
35	HWVKQTPGR	43	-0.35	-0.64	-0.52	-1.05	-0.11	-1.02
36	WVKQTPGRG	44	-1.3	2.26	1.93	1.24	2.31	1.22
37	VKQTPGRGL	45	-0.78	1.89	0.82	1.9	0.56	1.33
38	KQTPGRGLE	46	-0.35	-1.45	-1.83	-1	-0.9	-0.61
39	QTPGRGLEW	47	-0.28	-0.3	-0.29	0.22	-1.07	-0.45
40	TPGRGLEWI	48	-0.09	-1.98	-2.91	-1.66	-1.94	-1.72
Summarized Results				DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score				2.26	1.93	1.9	2.31	1.33
EpiVax Binding Data IC50 (nM)				192	4444	422	206	TBD
EpiVax Assessment				B	B	B	B	--

Motif is located at flank

More sensitive assay
(7 concentrations of peptide)
We observes two more
binders in original peptide

Optimized Peptide has a centered binding motif

With optimized version, we find **one more** binder and **stronger** affinities

Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors

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Rituximab/Infliximab Case Study – Centering Binding Motifs Example: RH 36-50

Original
EpiMatrix Cluster Detail Report
RH36-50 Cluster: 36

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
36	WVKQTPGRG	44	-1.3	2.26	1.93	1.24	2.31	1.22
37	VKQTPGRGL	45	-0.78	1.89	0.82	1.9	0.56	1.33
38	KQTPGRGLE	46	-1.63	-1.45	-1.83	-1	-0.9	-0.61
39	QTPGRGLEW	47	-1.3	-0.3	-0.29	0.22	-1.07	-0.45
40	TPGRGLEWI	48	-0.41	-1.98	-2.91	-1.66	-1.94	-1.72
41	PGRGLEWIG	49	-0.38	-1.19	-1.31	-1.56	-0.44	-0.59
42	GRGLEWIGA	50	0	-0.14	0.11	0.3	-0.04	0.34

Summarized Results	DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score	2.26	1.93	1.9	2.31	1.33
Publication Results	B	NB	NB	NB	NB
EpiVax Binding Data IC50 (nM)	1237	32143	7636	1424	36929
EpiVax Assessment	B	B	B	B	B
EPX cutoff: 1.64	TP	TP	TP	TP	FN

Centered
EpiMatrix Cluster Detail Report
RH36-50MOD Cluster: 33

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
33	NMHWVKQTP	41	-0.27	-1.53	-0.52	-1.08	-0.07	-0.55
34	MHWVKQTPG	42	-0.19	1.07	0.48	0.26	0.36	0.71
35	HWVKQTPGR	43	-0.35	-0.64	-0.52	-1.05	-0.11	-1.02
36	WVKQTPGRG	44	-1.3	2.26	1.93	1.24	2.31	1.22
37	VKQTPGRGL	45	-0.78	1.89	0.82	1.9	0.56	1.33
38	KQTPGRGLE	46	-0.35	-1.45	-1.83	-1	-0.9	-0.61
39	QTPGRGLEW	47	-0.28	-0.3	-0.29	0.22	-1.07	-0.45
40	TPGRGLEWI	48	-0.09	-1.98	-2.91	-1.66	-1.94	-1.72

Summarized Results	DRB1*0101	DRB1*0401	DRB1*0701	DRB1*1101	DRB1*1501
Maximum Single Z score	2.26	1.93	1.9	2.31	1.33
EpiVax Binding Data IC50 (nM)	192	4444	422	206	19360
EpiVax Assessment	B	B	B	B	B
EPX cutoff: 1.64	TP	TP	TP	TP	FN

Strong Binding Motif located at N-term flank

Four more binders observed with original peptide

Peptide modified to center the binding motif

Peptides bind to the same alleles at stronger affinities

Rituximab/Infliximab Case Study Example: Centering Binding Motifs – Second Example - IH 41-55

Original

EpiMatrix Cluster Detail Report

IH41-55 Cluster: 41

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
41	PEKGLEWVA	49	-0.44	-1.41	-1.46	-1.13	-0.6	-0.8
42	EKGLEWVAE	50	-0.66	-0.48	-0.32	-0.02	-0.97	-0.53
43	KGLEWVAEI	51	0.23	0.41	0.32	-0.05	-0.34	-1.63
44	GLEWVAEIR	52	0.17	-0.7	0.2	-0.37	-0.72	-0.77
45	LEWVAEIRS	53	0.12	0.23	0.44	0.5	1.01	0.88
46	EWVAEIRSK	54	-0.73	-0.98	-0.3	-0.33	-0.1	-1.08
47	WVAEIRSKS	55	-0.43	2.36	1.89	1.48	2.51	0.59
Maximum Single Z score				2.36	1.89	1.48	2.51	0.88
PUBLICATION RESULTS				2,760	10,000	22,727	11	238
				NB	NB	NB	B	NB
EpiVax Binding Data (nM)				136113	131563	51056	154	17373
EpiVax Assessment				NB	NB	B	B	B
EPX cutoff: 1.64				FP	FP	FN	TP	FN

Centered

EpiMatrix Cluster Detail Report

IH41-55MOD Cluster: 44

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*1101 Z-Score	DRB1*1501 Z-Score
44	KGLEWVAEI	52	0.05	0.41	0.32	-0.05	-0.34	-1.63
45	GLEWVAEIR	53	0.04	-0.7	0.2	-0.37	-0.72	-0.77
46	LEWVAEIRS	54	0.03	0.23	0.44	0.5	1.01	0.88
47	EWVAEIRSK	55	-0.16	-0.98	-0.3	-0.33	-0.1	-1.08
48	WVAEIRSKS	56	-0.43	2.36	1.89	1.48	2.51	0.59
49	VAEIRSKST	57	0.04	1.76	1.02	2.26	0.82	1.63
50	AEIRSKSIN	58	-0.15	-0.57	-1.56	-1.12	-0.07	0.04
51	EIRSKSINS	59	-0.21	1.45	1.66	1.44	1.32	1.29
Maximum Single Z score				2.36	1.89	2.26	2.51	1.63
EpiVax Binding Data (nM)				42787	60295	396	72	1265
EpiVax Assessment				B	B	B	B	B
EPX cutoff: 1.64				TP	TP	TP	TP	FN

Strong Binding Motif located at C-term flank

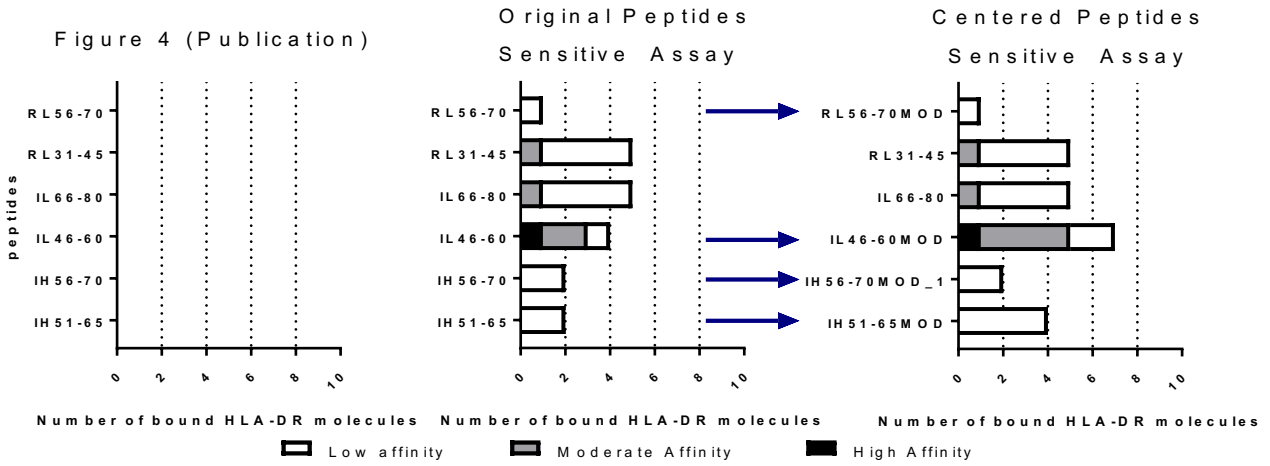
Two more binders observed with original peptide

Peptide modified to center the binding motif

Centered peptide binds to two more alleles (DR1 and DR4). Note that in centering, other motifs are introduced.

Rituximab/Infliximab Case Study – Centered Motifs

Improved Correlation between predicted and observed HLA binding



	n = 42
True Positive	0
False Positive	23
False Negative	0
True Negative	19
Positive Predictive Value	**0%**

	n = 42
True Positive	9
False Positive	14
False Negative	10
True Negative	9
Positive Predictive Value	**39%**

	n = 42
True Positive	12
False Positive	11
False Negative	12
True Negative	7
Positive Predictive Value	**52%**

Rituximab/Infliximab Case Study
Revised binding assay – Conclusions I

- Testing peptides (original design) using the competition based assay at EpiVax, we found that:
  - 19 peptide-allele pairs bound
    - Nine of these were predicted at top 5% (as predicted)
    - Eight of these were predicted at top 10%
    - Two were not predicted to bind (IH56-70 vs DR7 and IL66-80 vs DR15)
  - For the centered (mod) peptides
    - **IL46-60 and IH51-65 both show an increase** in the number of binders
    - RL56-70 and IH56-70 maintain the number of binders at one and two, respectively

- **Positive Predictive Value** of in silico predictions increases from **0% to 52%** after incorporating modifications

- While the assay is detecting **more predicted binding events**, there is a high false negative rate.
  - Majority of these observations are "false negatives" meaning bound but not predicted –
  - All were top 10% EpiMatrix predictions (we would generally not consider positive predictions)
  - However peptides scoring in this range do tend to bind fairly frequently when they occur within the context of an epitope cluster. Thus if we included top 10%, accuracy would be even higher.

Rituximab/Infliximab Case Study
Centered binding motifs– Conclusions II

- Similar to the previous results, we find that re-assaying original and centered peptides improves positive predictive value from **60% to 97%.**
  - We are seeing a higher rate of **predicted binders** becoming **observed binders** ✓
- However, accuracy remains similar to the publication results (62% to 64%). Why?
  - We are seeing a higher rate of *non-predicted binders* becoming *observed binders*.
    - Again, 50% of these peptide-allele pairs are predicted at the top 10%
    - Thus if we included top 10%, accuracy would be even higher.

Agenda

- Background
- HLA Class II Binding Assay: Principle and methods
- Rituximab/Infliximab Case Study
- Conclusions

Overall Conclusions from Hamze Repeat Assays Show that Binding Assays must be done more carefully

- We find that **centering binding motifs** in overlapping peptides yields **more binders** with **stronger affinities, improving association** of **in silico** predictions and **in vitro** findings**.**

- Careful attention should be taken to design peptides with optimal features, such as centered HLA binding motifs, before their usage in in vitro and in vivo experiments.

Agenda

- Core Concepts
- Immunogenicity
- Immunogenicity risk assessment tools
  - **Binding assays**
  - **T Cell Assays**
- iTEM – Personalizing Risk Assessment
- PANDA Case Study

How do you confirm T cell response?
T cell assays (done right!)

EpiVax

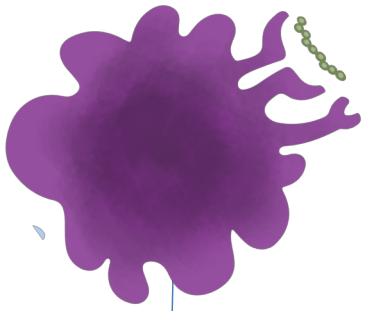
T effector assays for biologics –
Lots of variability in industry (need standardization)

T reg assays for biologics –
Tetanus Toxin Bystander Assay

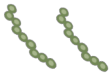
Variety of T cell assays used by Industry

In vitro immunogenicity Protocol or "IVIP"

MAPPS Assay



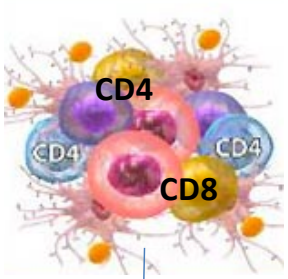
MHC immunoprecipitation



MS sequencing of peptides

Value added: peptide processing/competition

PBMC Assay - IVIP



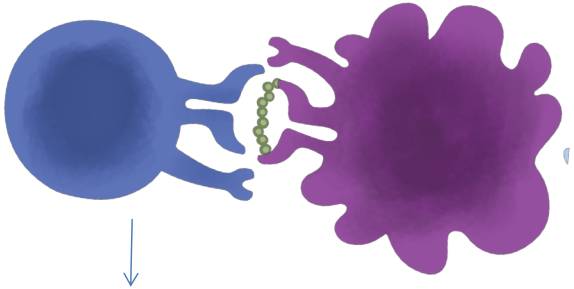
TNFa, IL2, IFNg

Luminex/Elispot/ICS /Proliferation

Validation of immunogenicity/ high sample numbers;; low sensitivity for primary responses

DC/T cell Assay

Generate moDC



TNFa, IL2, IFNg

Luminex/Elispot/ICS /Proliferation

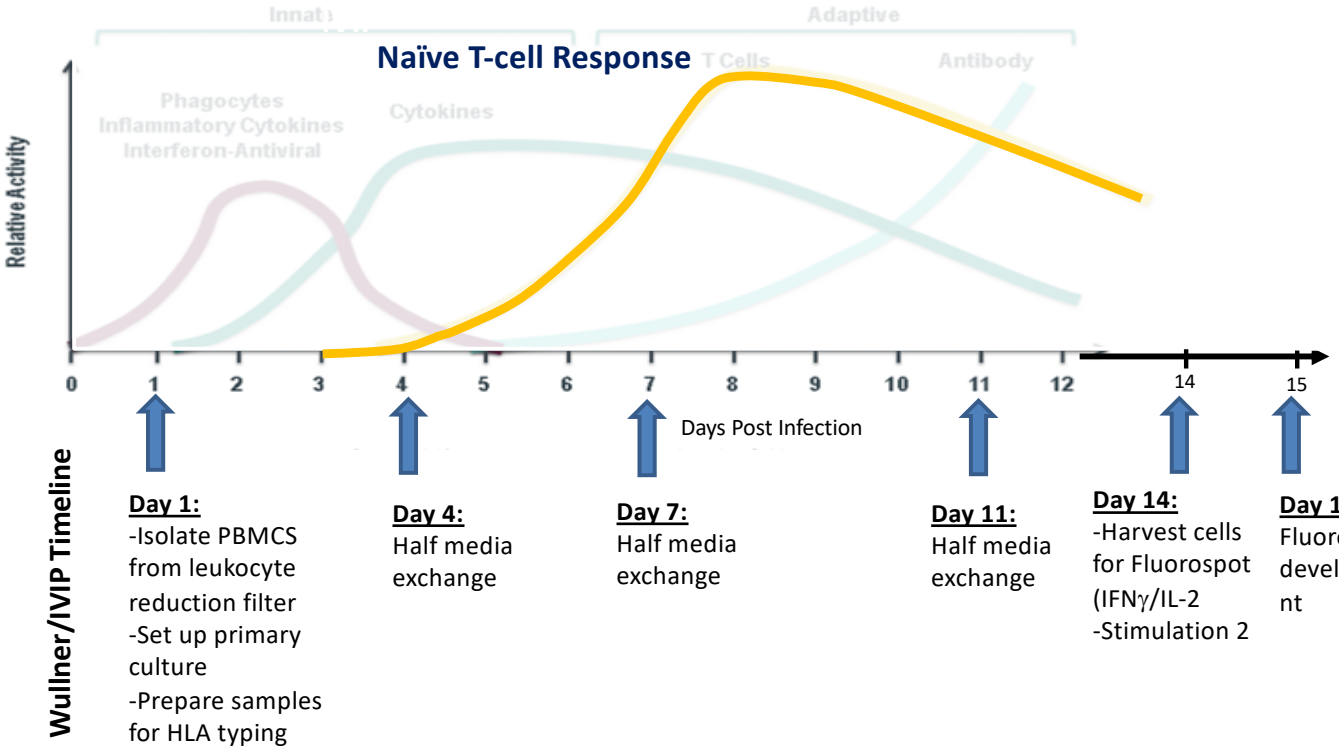
High Sensitivity, Technically Complex

*I*n *v*itro *I*mmunogenicity *P*rotocol (IVIP)

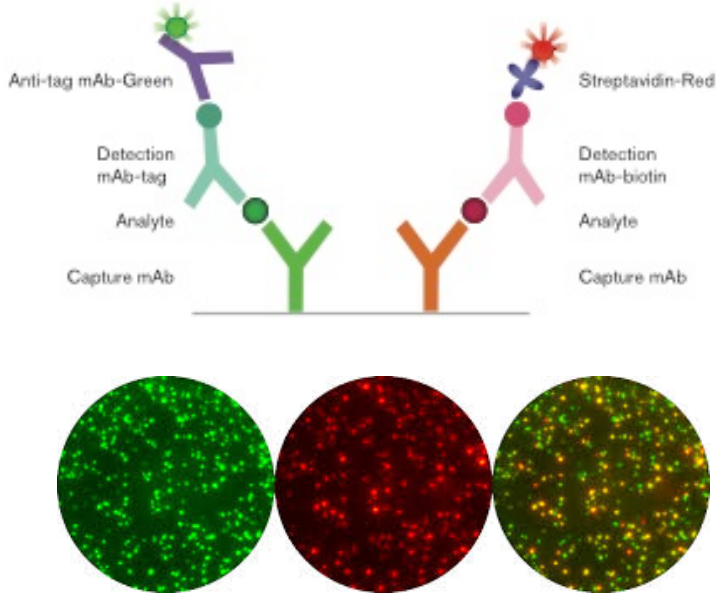
- **The ability of the test article (new Generic) and the RLD to stimulate a *de novo* T-cell response is compare to several controls** including HSA (protein neg control), KLH (protein positive control) and a CEFT (protein pool positive control).

- **14 days post exposure, cells are harvested and plated into precoated IFNγ ELISpot plates.** Cells are restimulated and incubated overnight. On day 15, ELISpot plates are developed and sent to Zellnet Consulting Inc. for blind, independent analysis.

Timeline of the "Naïve" immune response



ELISpot Assay
Concept and Read Out



+ Enables detection of low-frequency cells

+ Antigen specificity of response

- Cannot easily distinguish types of cells

- Limited to 1 or 2 target cytokines per assay

How do you confirm T cell response?
T cell assays (done right!)

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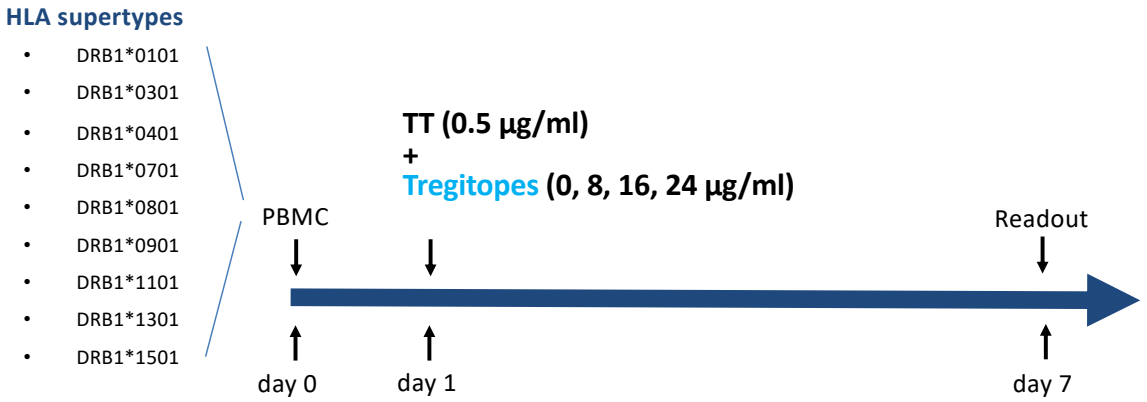
T effector assays for biologics –
In vitro immunogenicity Protocol or IVIP

T reg assays for biologics –
Tetanus Toxin Bystander Assay

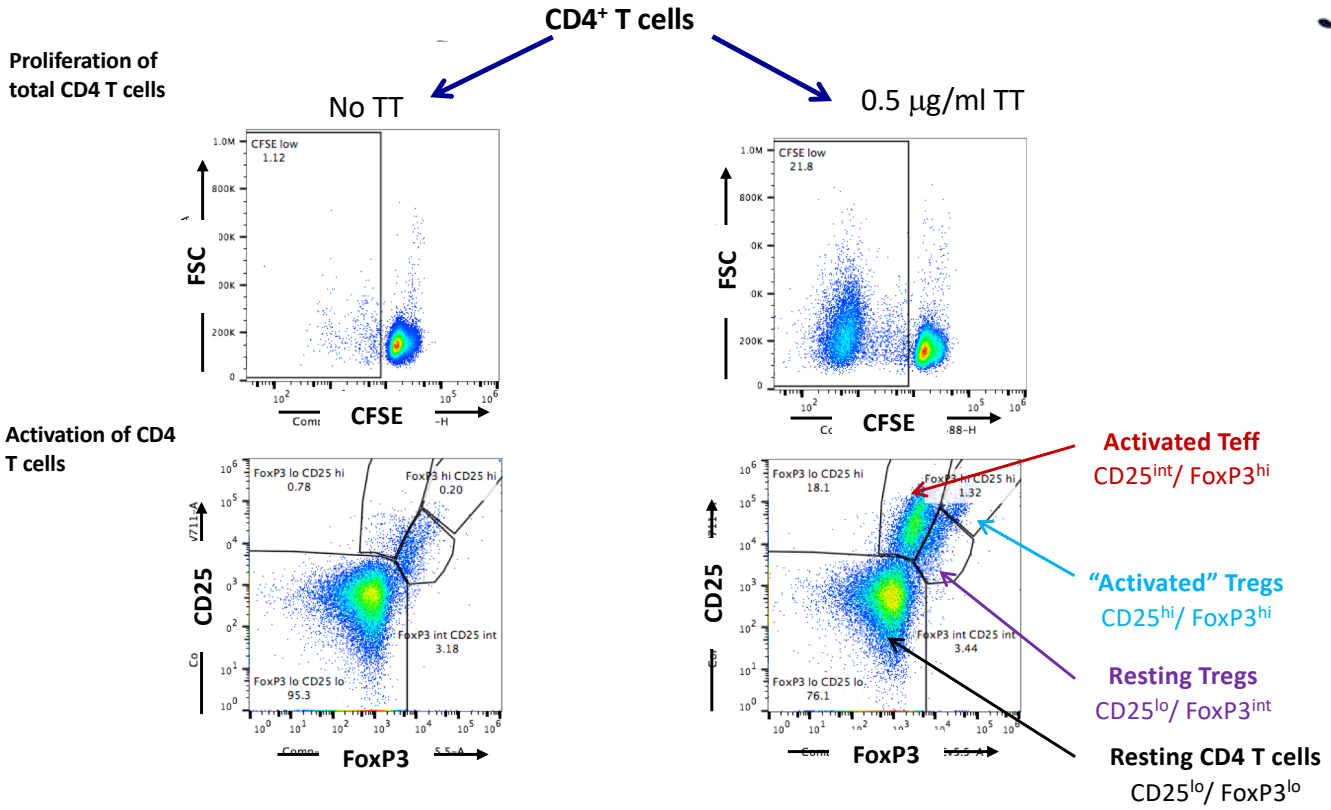
In Vitro Assay – Treg Tregitope Confirmation
Tetanus Toxin Bystander Suppression Assay



- Proliferation and activation of Teff (CD45RA, CCR7, CD25)
- Proliferation and activation of Treg (CD25, FoxP3, CD127)

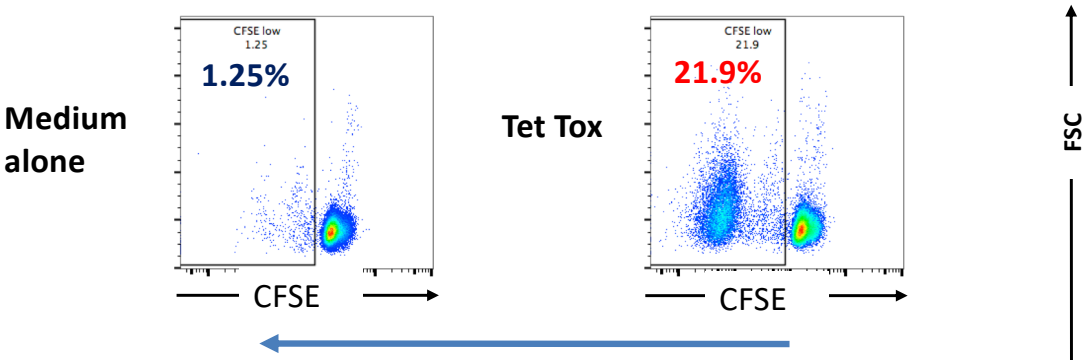
Tregitopes are tested alone or in combination

CD4 effector T cell response
(we are measuring Teffector Immune response to Tet Tox)



By Flow - CD4 T cell TT recall response

Proliferation of total CD4 T cells



Proliferation of T cells is shown by shift to the left

.

Factor V has a Tregitope (Amy Rosenberg/Bill Martin) Unpublished assays by Eduardo Guillen/Sandra Lelias

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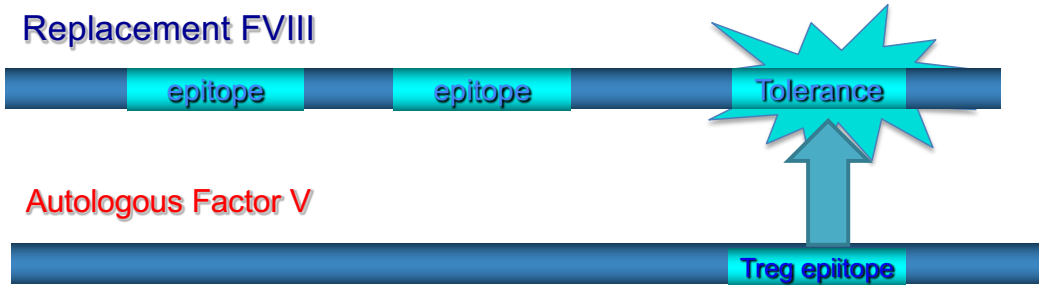
Other Autologous Proteins with Similar (Homologous) Epitopes may be Tolerogenic



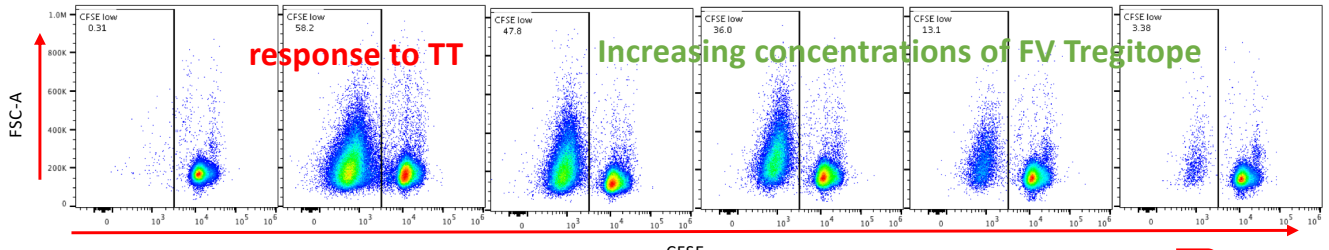
We ask:

- *Do Autologous T reg epitopes (in FV) regulate immune response to FVIII?*
- *Could these autologous Treg epitopes be used to induce FVIII-specific tolerance?*
- ***We think YES***

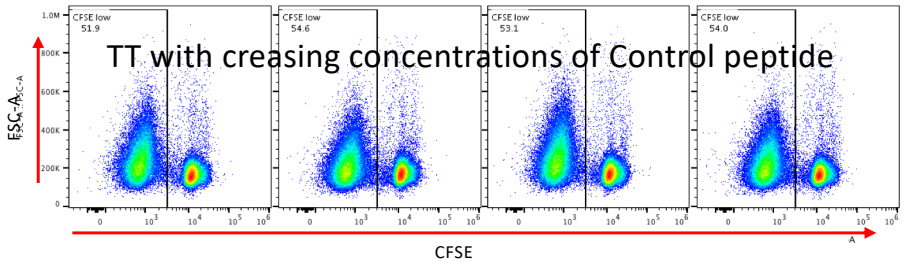
Tetanus Toxin "Bystander Suppression" assay Shows Factor V Tregitope suppressing TTox response

Tregitope co-incubation demonstrates bystander suppression of CD4 effector response

Tregitope concentration at arrow			↴	↴	↴	↴
TT (µg/ml)	0	0.5	0.5	0.5	0.5	0.5
TEST Tregitope	0	0	5	10	15	20



CONTROL Peptide	0	0	5	10	15	20



Factor V has a Tregitope (Amy Rosenberg/Bill Martin)
Unpublished assays by Eduardo Guillen/Sandra Lelias

T cell assays

- T effector assays can be performed on “self” proteins and peptides.
- **In vitro assays – see publications by Wullner et al. and Jawa et al.**

- Treg assays can be used to validate in silico predictions.
- **Publications are forthcoming from the EpiVax Tregitope group**

What about Peptide Elution? (Rotzche and Falk)

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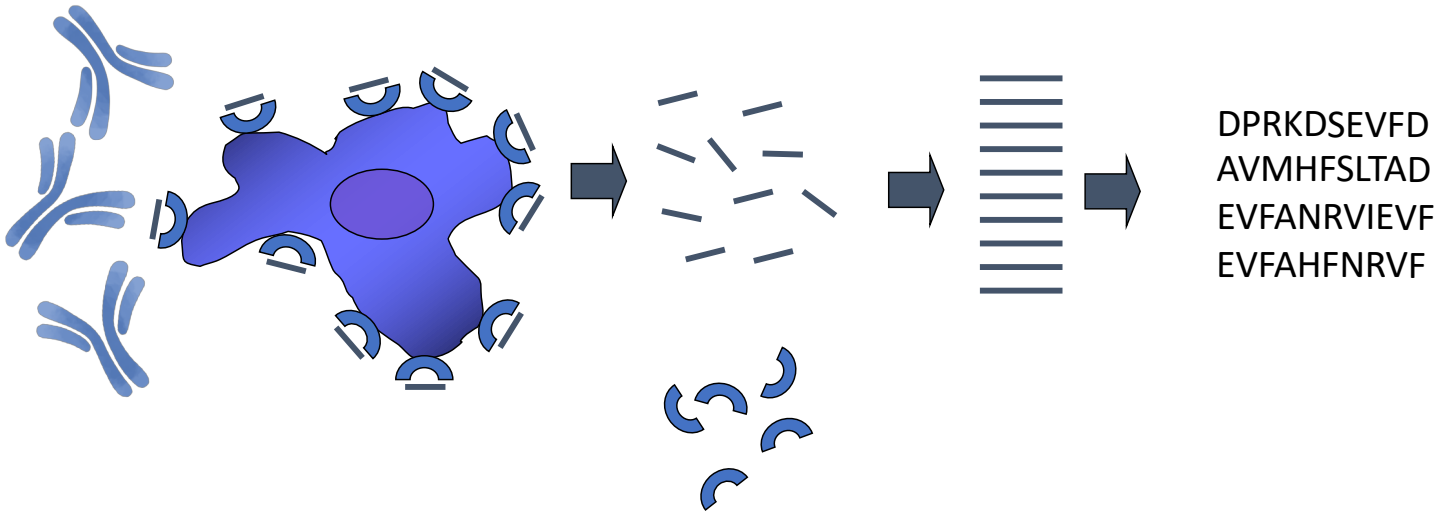
MAPPS ASSAY

Pulse, Elute, Sequence, Align. (lots of work!)



MAPPS
– Data courtesy of Nobuo Sekiguchi Chugai

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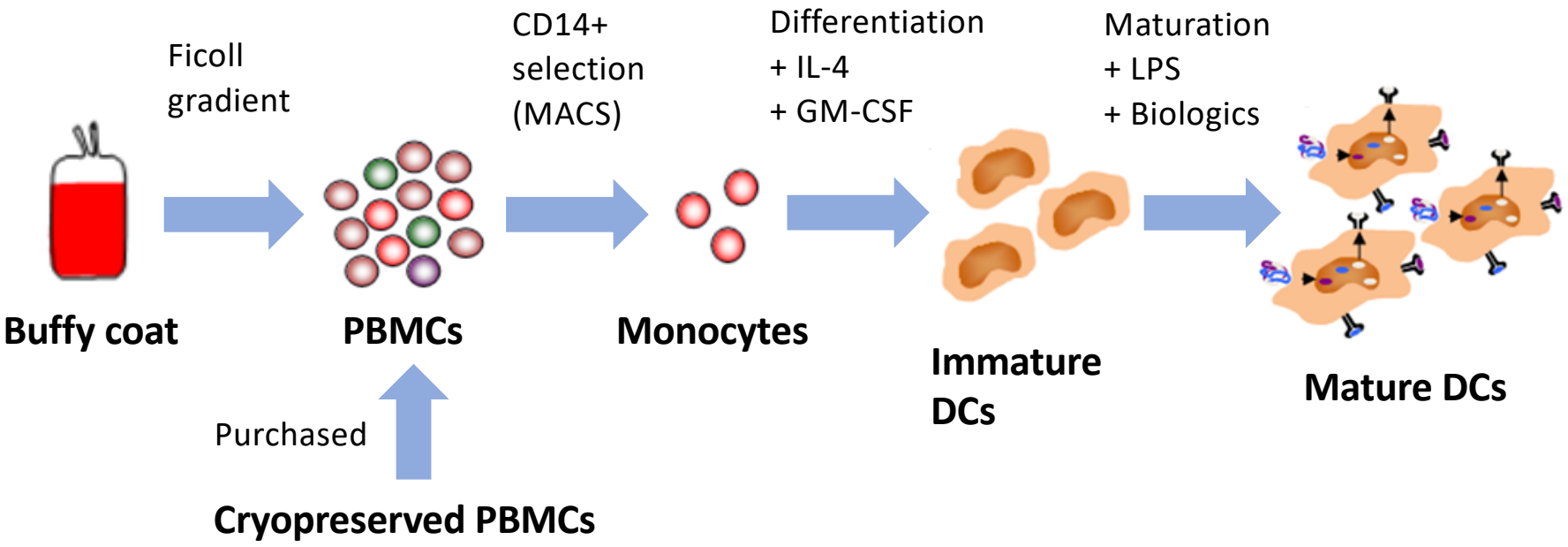
Report

MHC-associated peptide proteomics enabling highly sensitive detection of immunogenic sequences for the development of therapeutic antibodies with low immunogenicity

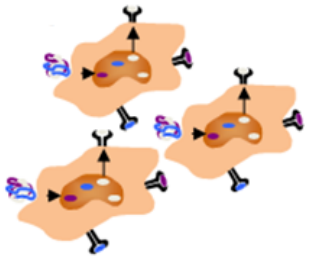
Nobuo Sekiguchi, Chiyomi Kubo, Ayako Takahashi, Kumiko Muraoka, Akira Takeiri, Shunsuke Ito, ...show all

Pages 1168-1181 | Received 19 May 2018, Accepted 29 Aug 2018, Accepted author version posted online: 10 Sep 2018, Published online: 01 Oct 2018

Sekiguchi (Chugai) MAPPs procedure (1)



Sekiguchi (Chugai) MAPPs procedure (2)



Mature DCs

Lysis

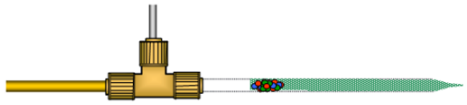
Peptide-HLA-DR complex

Immuno-precipitation

Beads coupled with anti-HLA-DR

Wash & Acid elution

Peptide mixture



Nano reverse phase HPLC

Orbitrap MS

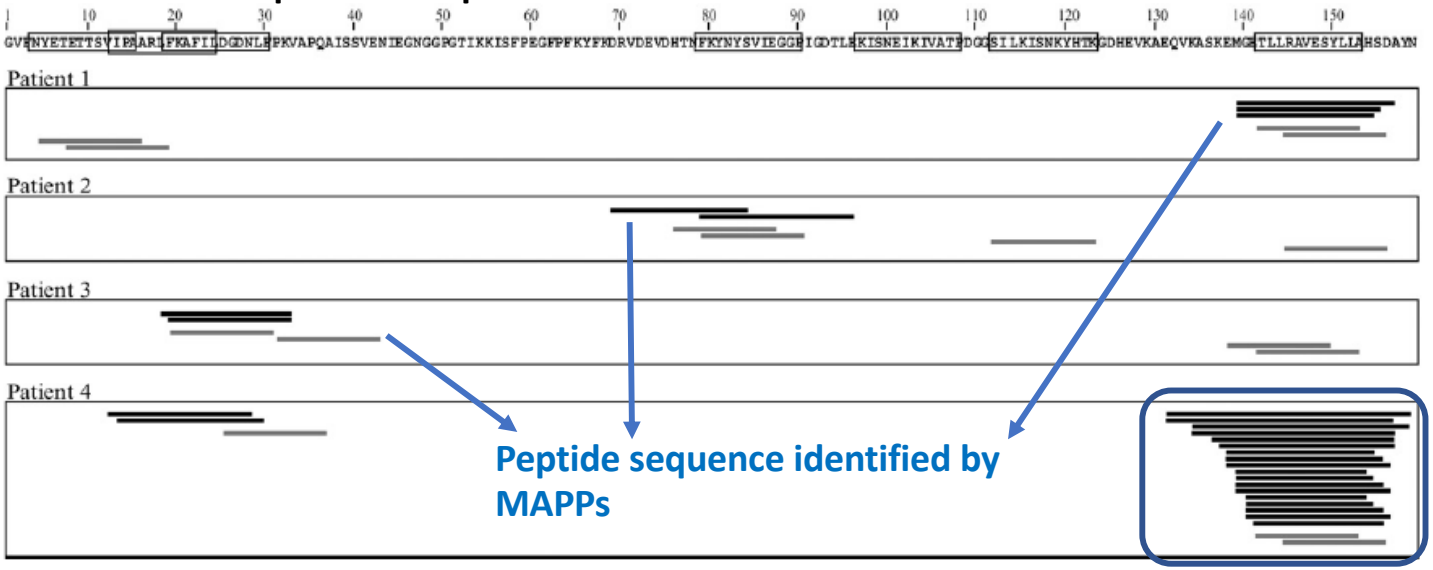
- Accurate mass
- MS/MS

Peptide ID by DB analysis

MAPPs data image

Example of Bet V1 (birch pollen allergen)

Amino acid sequence of a protein evaluated

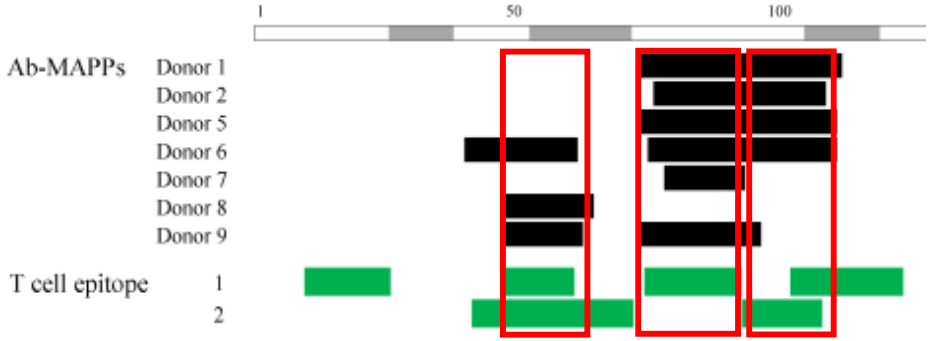


Multiple length variants sharing the same core sequence

Mutschlechner S et al., J Allergy Clin Immunol 2010, 125:711

Comparison of MAPPs data with T cell epitopes reported

Infliximab VH region



Infliximab VL region

Sekiguchi N et al., mAbs in press

MAPPs

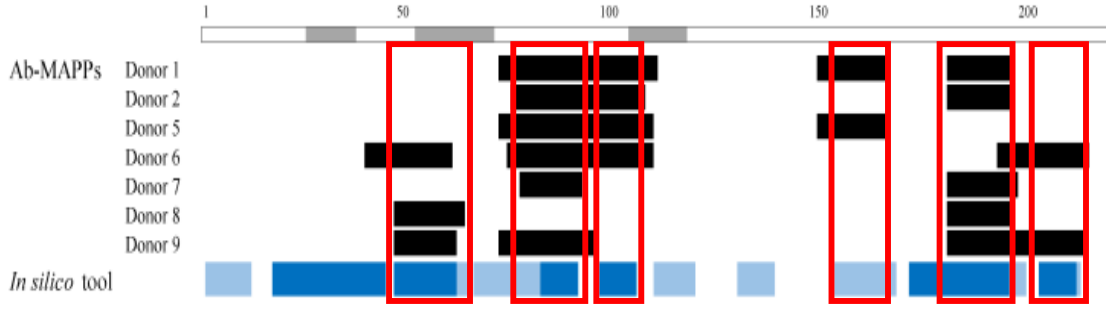
T cell epitopes reported

1) Hellendoorn K et al., US patent US7754853B2
2) Hamze M et al., Front Immunol 2017, 8:500

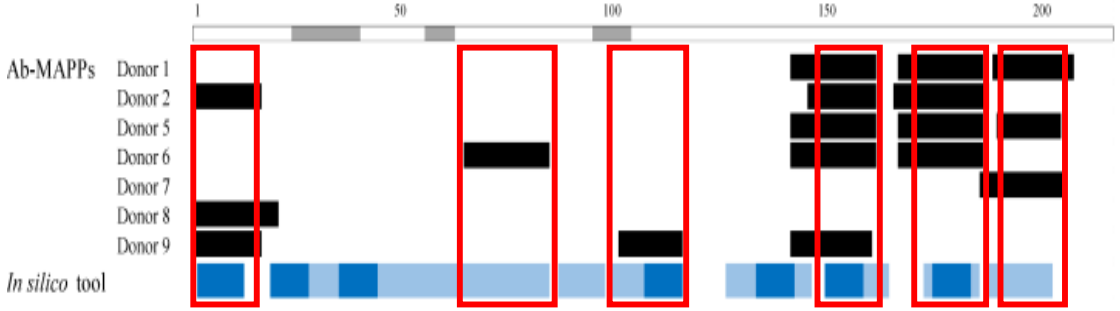
Many of the seq regions identified by MAPPs corresponded to T cell epitopes reported in infliximab.

Comparison of MAPPs data with in silico prediction

Infliximab heavy chain



Infliximab light chain



MAPPs

EpiMatrix

Hit in more than 2 alleles

Hit in 1 allele out of 4 allele types

Core seqs for almost all of the peptides identified by MAPPs overlapped with hits predicted in silico.

Sekiguchi N et al., mAbs in press

Differences between MAPPS and In Silico are due to Individual vs. Population approach.
In silico tools predict for populations; MAPPS measure allele-by-allele or personal peptide repertoire

Secukinumab Case Study: Karle et al (Novartis)
MAPPS/T cell assays vs. In Silico Prediction

EpiVax

MAbs. 2016 Apr; 8(3): 536–550. PMCID: PMC4966846
Published online 2016 Jan 28.
doi: 10.1080/19420862.2015.1136761

Secukinumab, a novel anti–IL-17A antibody, shows low immunogenicity potential in human in vitro assays comparable to other marketed biotherapeutics with low clinical immunogenicity

Anette Karle, Sebastian Spindeldreher, and Frank Kolbinger

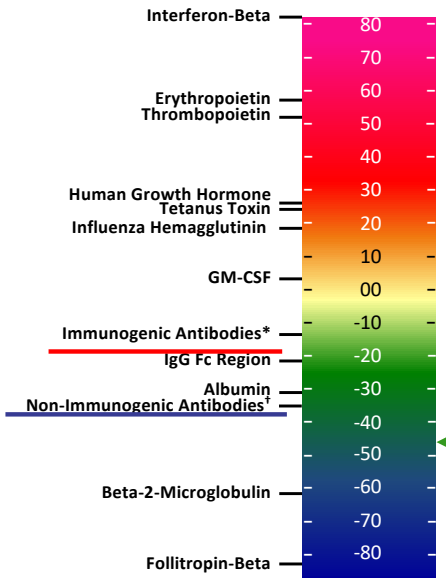
Author information ▶ Article notes ▶ Copyright and License information ▶

Annette Karle – Months of hard work!

MAPPS assays give patient-level data.

In silico analysis is fast and gives a very good assessment of immunogenicity risk.

In silico data can also give population-level risk.



Secukinumab <15 minutes

MAPPS vs. ISPRI -Predicted Epitopes
MAPPS does not define PHENOTYPE of response

Green – Tregitope
or High JanusMatrix (Human) Score

Green Box: JanusMatrix ≥3 or Tregitope
Red Box: JanusMatrix <3
Putative Treg or tolerated epitope

All positions are relative

For all antibodies:
IgG1: HC backbone
Ig Kappa: LC backbone

MAPPS and In Silico – *Different Timelines*
Complementary ? (doesn't identify Tregitopes)

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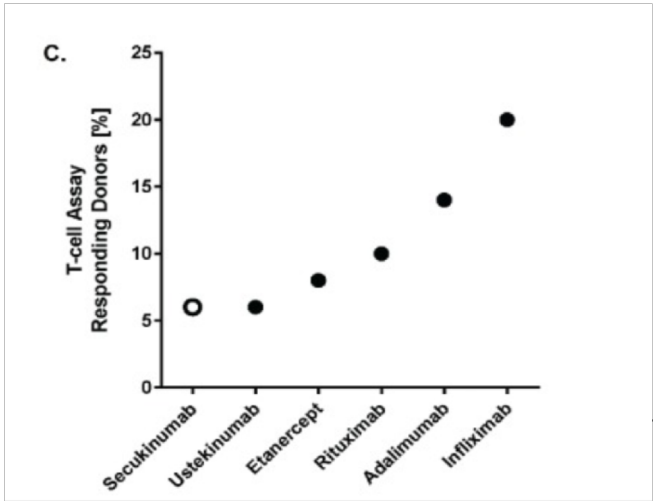
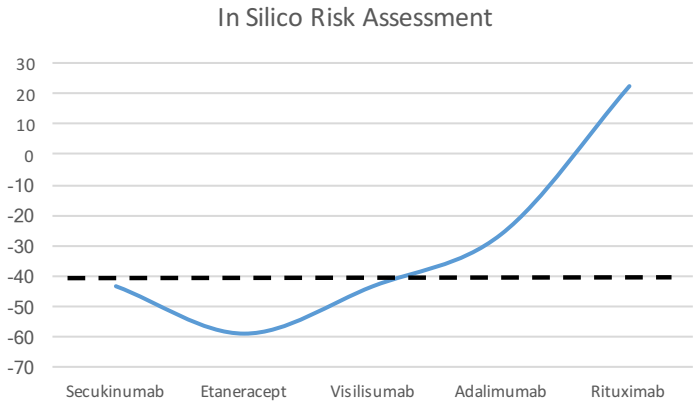
MAPPS – **Months of work?**



From: Spindeldreher et al., 2018 Dermatol Ther 8:57-68

ISPRI in silico assessment **60 minutes**



From: ISPRI Website
Assay performed in Lisbon, 2017

New: J-iTEM - in vitro validation of Janus adjusted Individualized T cell epitope Measure

- **Janus-iTEM** (J-iTEM)
  - **HLA-restricted T effector epitope content (EMX)**
  - **Putative HLA-restricted Treg (JMX)**
  - **for each individual HLA allele**

- **Combines Teff + Treg in one score**

JanusMatrix + iTEM = iTEM – new data

Adjusting for regulatory epitopes and HLA to personalize immunogenicity

1 + 1 - regulatory T cell epitope = response

T cell response depends on:

T cell epitope content – Tregitope content + HLA of subject

Further characterization of T cell epitope content leads to more accurate prediction of immunogenicity

HLA Restricts Immune Response (Personalizing Risk Assessment) / iTEM

Protein Therapeutic:

1 + 1 + 1 = Response

T cell response depends on:

T cell epitope content + **HLA of subject**

- protein immunogenicity can be ranked

De Groot A.S. and L. Moise. Prediction of immunogenicity for therapeutic proteins: State of the art. Current Opinions in Drug Development and Discovery. May 2007. 10(3):332-40.

Different HLA, Different Binding Pockets

HLA-DR B*0101

HLA-DR B*0301

iTEM to find HLA restricted tolerance
+ JanusMatrix to find Treg/Tolerated epitopes = J-iTEM

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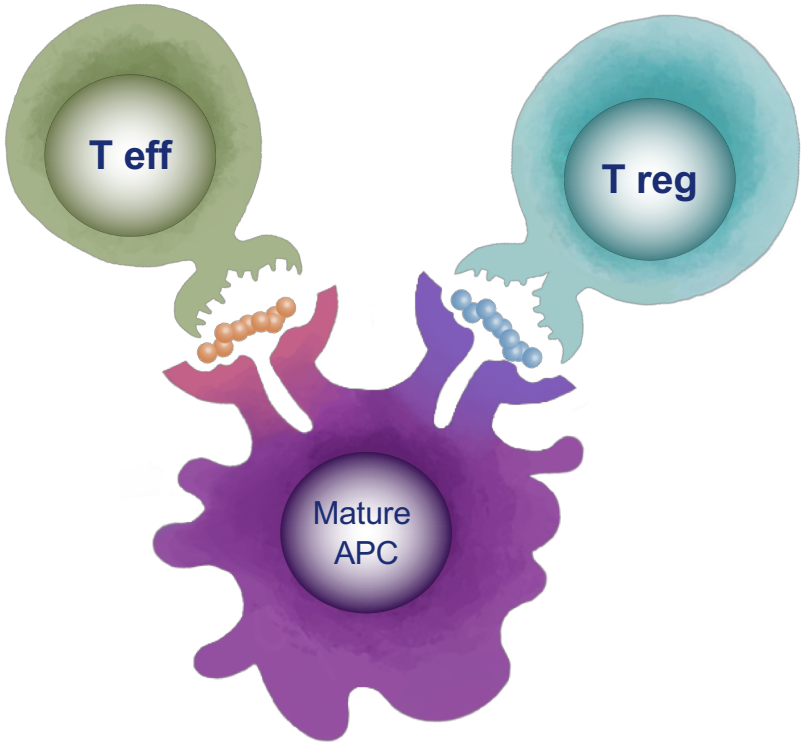

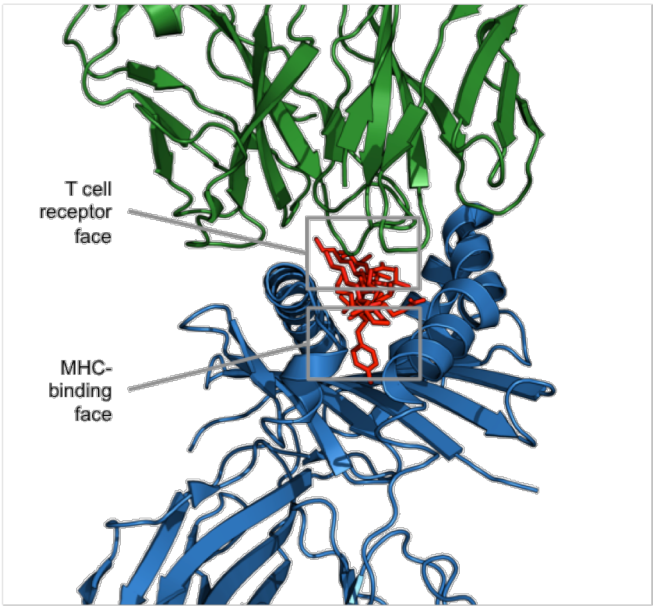

J-iTEM
Janus adjusted Individualized T cell epitope Measure

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For each volunteer, we calculated a J-iTEM score for that peptide.
Example shown for volunteer XXX for peptide 48

DRB1*0701 iTEM = 1.7 + (1.65 / 2) = 2.52

DRB1*0901 iTEM = 1.66

0701/0901 iTEM = 2.52+1.66= 4.18

These two hits each have 2 or more cross-conserved hits with the human genome i.e. JMX=2; could be tolerated/actively tolerogenic

To calculate J-iTEM, we remove these hits from the calculation, but deductions are maintained.

DRB1*0701 J-iTEM = 1.7 + ~~(1.65 / 2)~~ = 1.7

DRB1*0901 J-iTEM = ~~1.66~~

0701/0901 J-iTEM = 1.7

Protein ID	Protein D					DRB1 0101	DRB1 *0301	DRB1 *0401	DRB1 *0701	DRB1 *0801	DRB1 *0901	DRB1 *1101	DRB1 *1301	DRB1 *1501
RH5_305-326 (Peptide 48)						DB Ver: September 03, 2017					EpiMatrix Ver: 1.2			
						DRB1 0101	DRB1 *0301	DRB1 *0401	DRB1 *0701	DRB1 *0801	DRB1 *0901	DRB1 *1101	DRB1 *1301	DRB1 *1501
						-0.61	-0.24	-0.78	-1.15	0.58	-1.02	0.67	-0.01	-0.6
		306	EYNTKKKKL		0	0.46	-0.18	-0.64	0.75	-0.87	0.65	0.22	0.85	0.54
		307	YNTKKKKLI		7	1.19	1.75	-0.11	1.65	2.8	1.66	.89	2.38	0.82
sp|P56559|ARL4C_HUMAN	ADP-ribosylation factor-like protei					1.53	1.21	1.94	1.81	0.93	1.11	.72	1.18	0.38
sp|A2A2Z9|AN18B_HUMAN	Ankyrin repeat dom					2.65	1.27	1.76	1.89	2.26	1.34	.72	1.69	1.57
sp|Q8IVF6|AN18A_HUMAN	Ankyrin repeat don					2.65	1.27	1.76	1.89	2.26	1.34	.72	1.69	1.57
sp|Q9P2D7|DYH1_HUMAN	Dynein heavy chai					1.07	1.42	0.7	1.87	1.3	1.9	.24	1.59	1.9
sp|Q6ZWJ1|STXB4_HUMAN	Syntaxin-binding pr					1.48	0.79	0.6	2.18	1.35	0.83	.34	1.21	1.37
sp|P62487|RPB7_HUMAN	DNA-directed RNA					0.83	0.78	0.32	1.95	0.82	1.68	.11	1.18	0.24
sp|Q15911|ZFHX3_HUMAN	Zinc finger homeob					0.92	1.22	-0.1	0.87	2.42	0.11	.36	1.64	0.67
						-0.56	0.64	-0.44	-0.65	1.59	-0.79	1.32	1.23	-0.08
						-0.49	-0.28	-0.94	0.11	1.26	-0.65	0.26	0.15	-0.02
						-0.12	0.33	-0.68	1.7	0.97	-0.09	0.1	0.99	-0.39
		311	KKKLIKCIK		0	0.67	0.11	-0.2	0.03	0.92	-0.43	1.98	0.21	0.59
		312	KKLIKCIKN		0	0.58	-0.3	1.5	0.74	0.41	0.14	0.76	0.03	0.6
						-1	-0.47	-0.5	0.44	1.27	0.25	0.3	0.2	-1.24
						-0.52	1.52	0.06	-0.56	2.94	0.53	0.72	0.86	0.21
sp|Q86Y37|CACL1_HUMAN	CDK2-associated					1.52	2.08	1.38	2.41	2.09	1.8	1.65	1.72	1.26
						1.94	0.6	1.99	1.57	0.61	1.15	1.4	0.97	1.28
						-1.41	-0.43	-1.58	-0.64	1.16	-1.03	-0.78	-0.43	-1.27
						-0.62	0.61	0.01	0.76	0.33	0	0.64	-0.17	-0.64
						1.02	-0.2	1.08	1.09	-0.06	0.6	0.14	0.79	0.46

USAID analysis for Malaria Study (Leidos/Oxford

J-iTEM Clinical Results – Peptide-based Assay Data T cells / Oxford (Malaria Vaccine)

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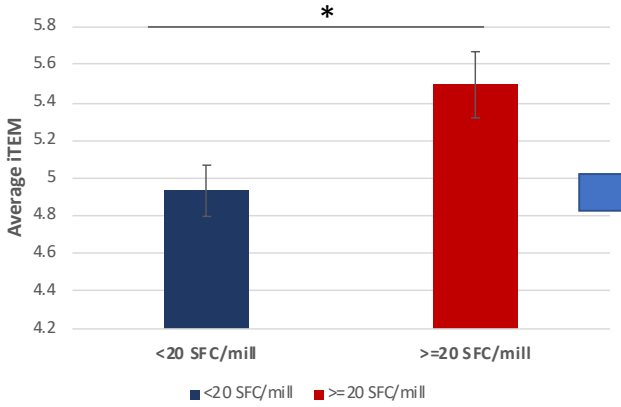
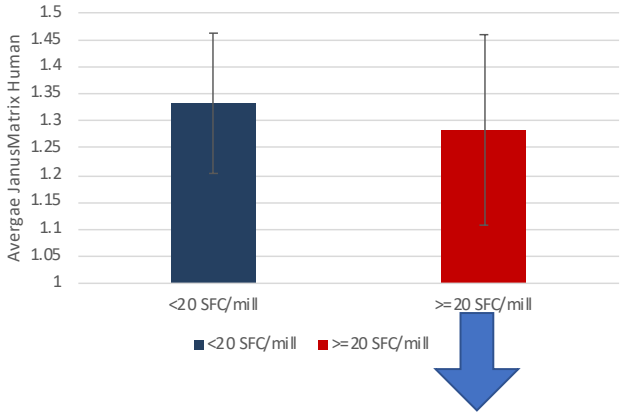
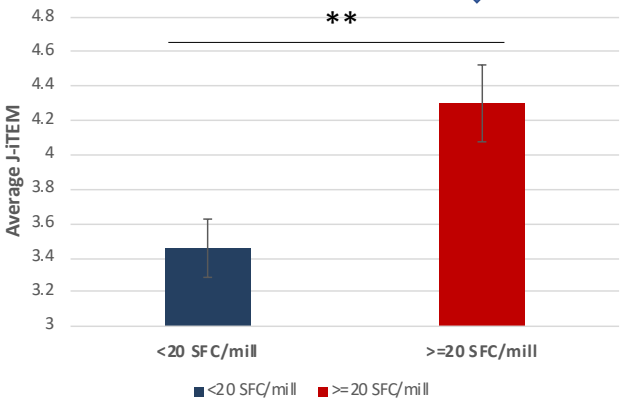
Standard iTEM





JMX

Without iTEM



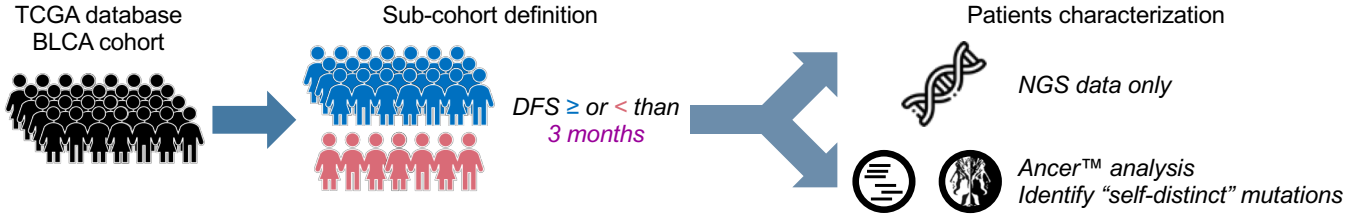
J-iTEM

iTEM + JMX

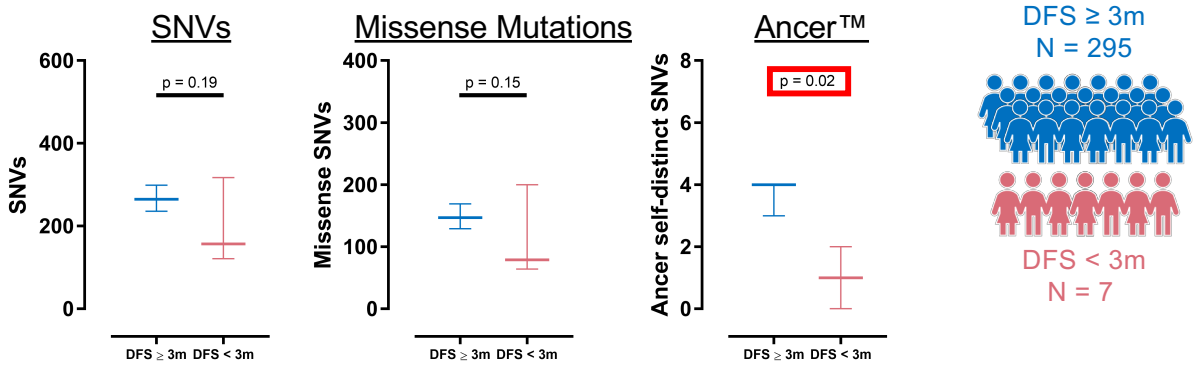
- **288 T cell assays. # <20SFC = 168; # ≥20SFC = 120**
- iTEM scores of **negative responses** <20 are lower than iTEM scores of **positive responses** ≥ 20. p<0.05
- **JanusMatrix** scores of **negative responses** <20 **are higher** than JMX scores of positive responses ≥ 20. (ns)
- **Combined J-iTEM scores of responses <20 are significantly lower than J-iTEM scores of responses ≥ 20. p<0.01**
- **Simon Draper, Oxford**

UNIVERSITY OF OXFORD

Can J-iTEM help explain the prognosis of bladder cancer patients from the TCGA?



Only Ancer™ can significantly separate patients with DFS greater or lower than 3 months. Patients with a DFS greater than 3 months have higher number of self-distinct mutations (i.e. mutations generating epitopes that have a non-self TCR face). Similar results are obtained using a 6-month DFS cutoff.



Median with 95% CI. Differences evaluated by Mann-Whitney test.

J-iTEM Ancer™ -selected CT26 murine neoantigen peptides personalized vaccine

EpiVax

CT26
Colon carcinoma

Ancer™

Mutation Catalog (Literature, Vendor) → Neo-Epitope Analysis → Neo-Epitope Selection → Vaccine Formulation → Immunogenicity Vaccine Efficacy

Input data
- 3,267 variants
- 1,787 missense SNVs
- 378 candidate SNVs after QC and filtering

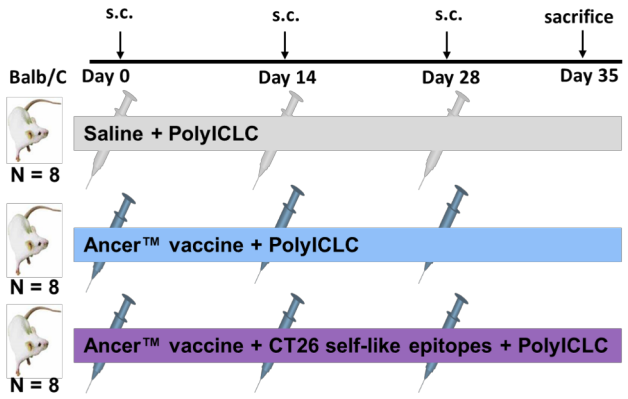
Analysis
- 135 candidate peptides
- 27 putative Class II Treg epitopes filtered out

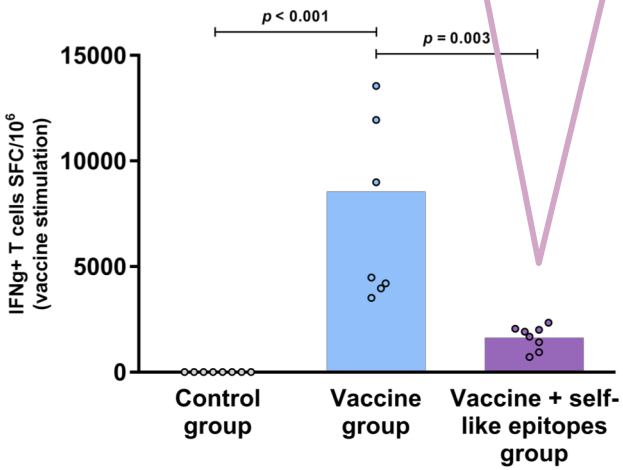
Selection
- 20 candidate peptides

Peptide vaccination

Ancer™ selected CT26 Treg neoantigen peptides reduce neoantigen vaccine immune responses

5-fold reduction of IFNγ responses with co-administration of CT26 self-like neo-epitopes



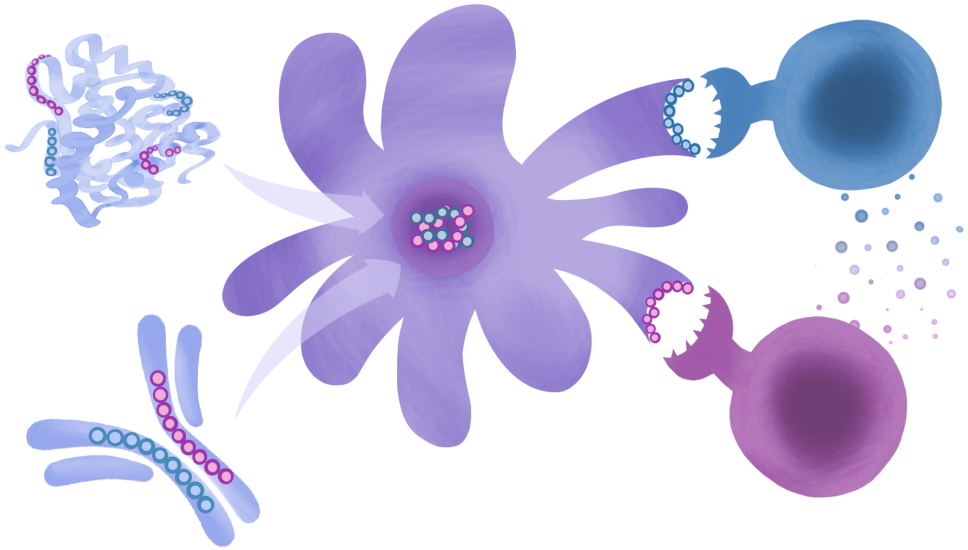


Preliminary Immunogenicity Results:

Splenocytes were collected at day 35 and restimulated in IFNγ ELISpot assay with the 20 vaccine peptides. Strong IFNγ responses are observed in the vaccinated group after restimulation compared to the control group. Co-administration of Ancer™-derived CT26 self-like neo-epitopes identified with JanusMatrix™ significantly reduce IFNγ responses by 5-fold.

Questions?

EpiVax



EpiVax 20 YEARS Fearless Science

Agenda

Babraham Immunogenicity Seminar 2019 January 23, 2019 — Babraham Research Campus	
Time	**Topic**
10:00	**Arrival and Coffee**
10:30	Immunogenicity and Tolerance: Setting the Stage
11:30	Evolution of In Silico Tools for Predicting and In Vitro Assays for Validating T-cell Response
12:30	**Lunch**
12:45	Demonstration of In Silico Immunogenicity Assessment Tools
13:55	Closing Remarks

In Silico