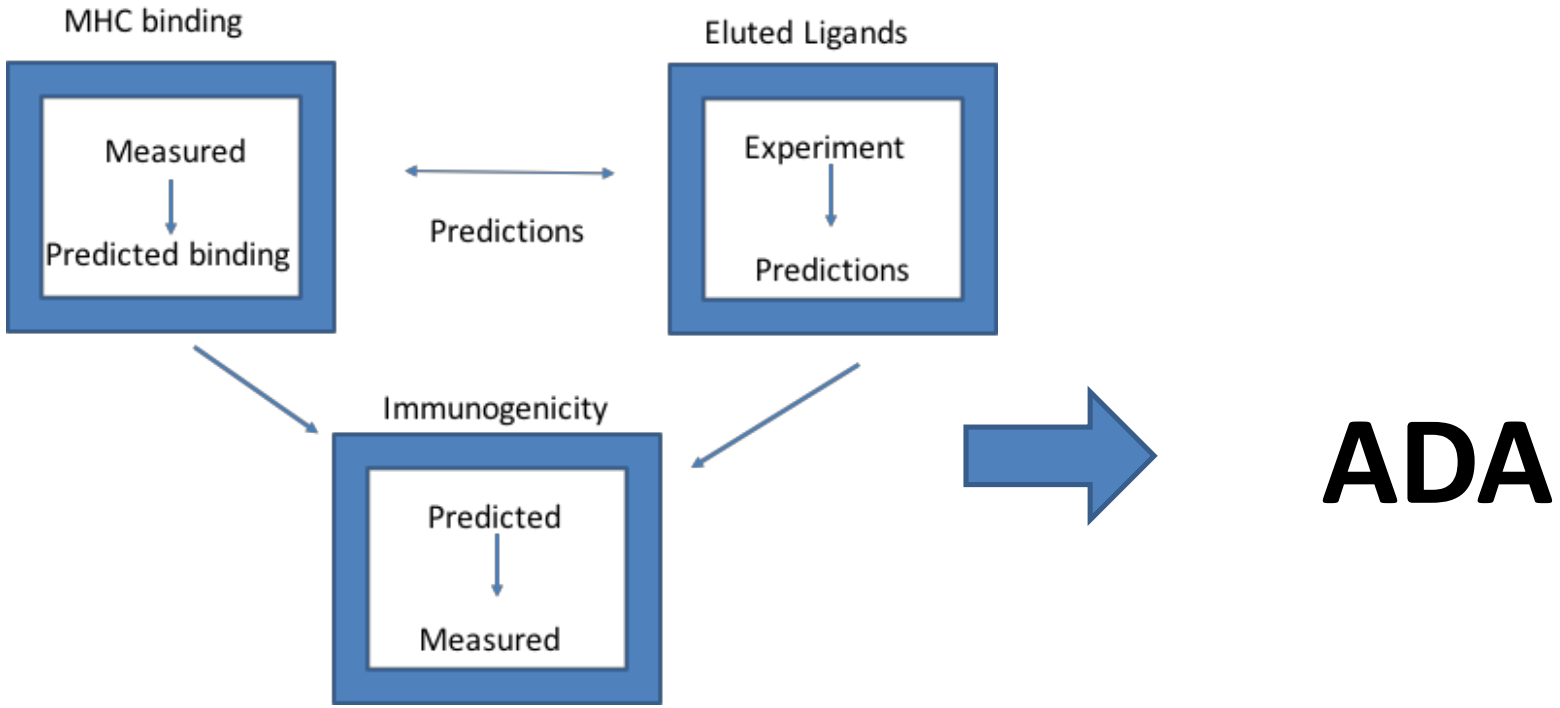
MHC binding, eluted ligands and immunogenicity; benchmarking testing and predictions

Non-clinical Immunogenicity Assessment of Generic Peptide Products: Development, Validation, and Sampling workshop. FDA January 26, 2021

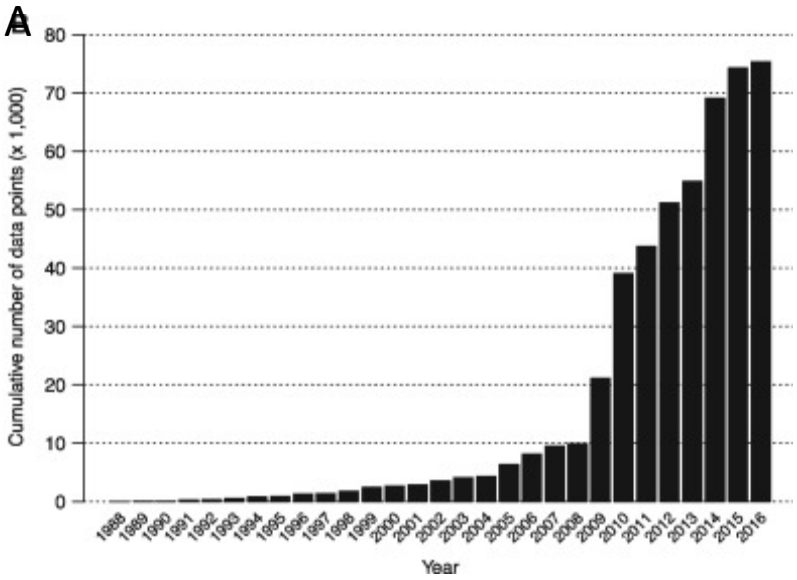
How effective is testing/predictions to ultimately predict drug immunogenicity?

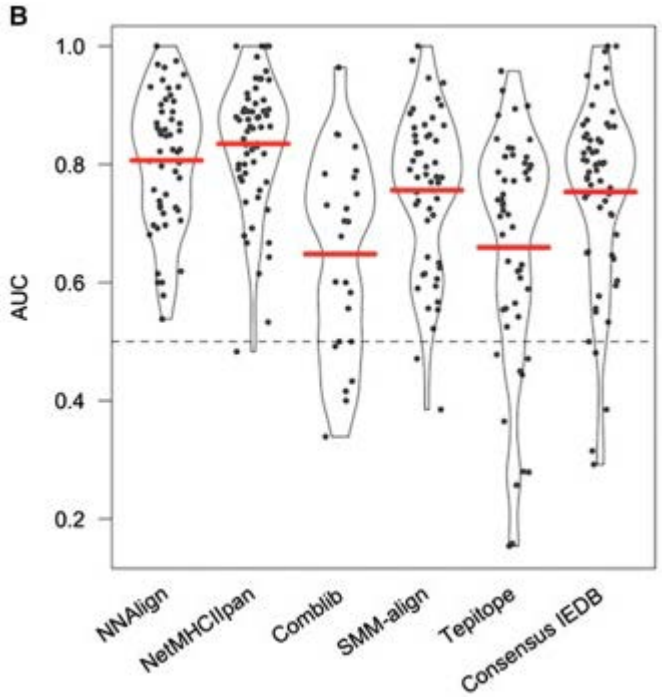


An automated benchmarking platform for MHC class II binding prediction methods

Massimo Andreatta, Thomas Trolle, Zhen Yan, Jason A Greenbaum, Bjoern Peters, Morten Nielsen ✉

Bioinformatics, Volume 34, Issue 9, 1 May 2018, Pages 1522–1528, https://doi.org/10.1093/bioinformatics/btx820



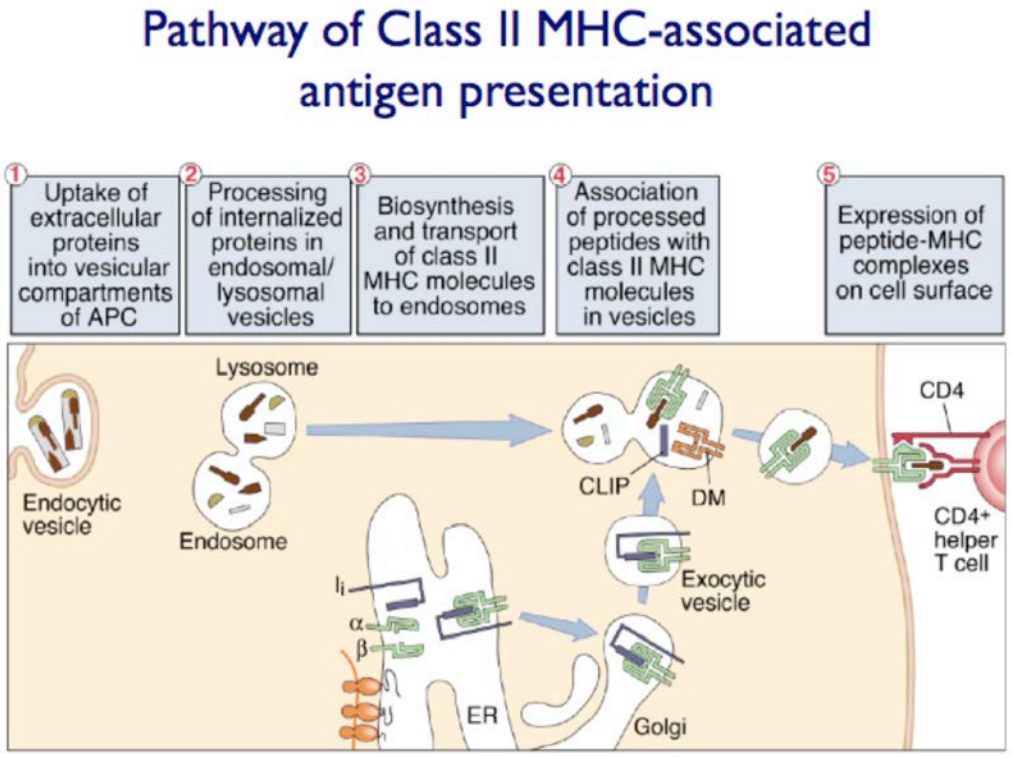


Conclusions:

- Automated objective benchmarking established
- Performance still lower than class I, but improving and over 0.8 for the best methods

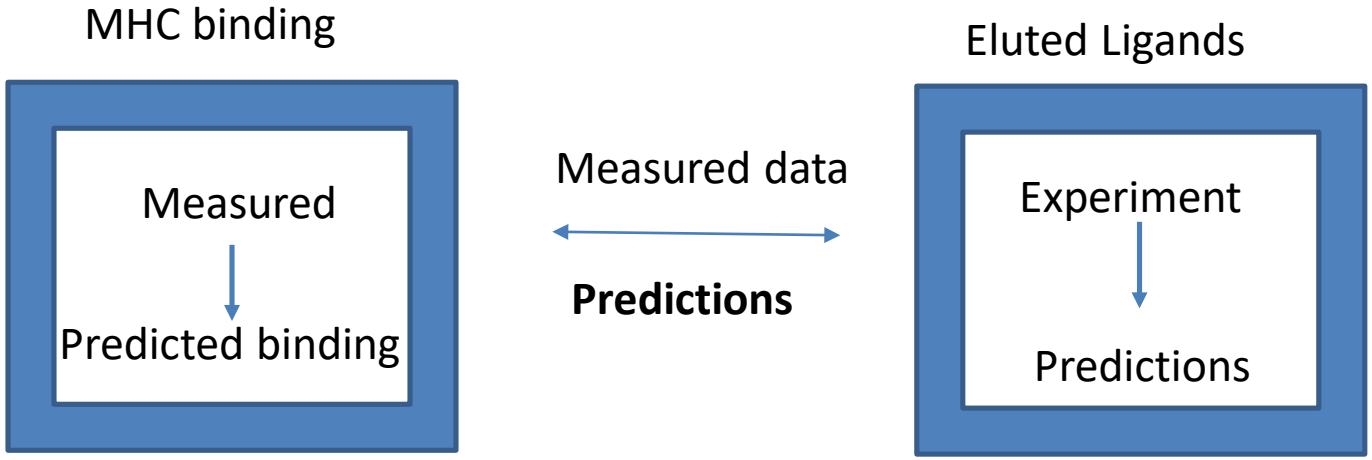
Naturally ligand data offers a different learning opportunity

- The MAPPS assay provide sequences from eluted peptides naturally bound to HLA
- This can account for influences of processing , beyond just HLA binding
- Does MAPPS predict immunogenicity?
- How do the peptides identified by the MAPPS assay correlate with those identified by binding predictions?



Garde C. Immunogenetics. 2019 Jul;71(7):445-454. PMID: 31183519

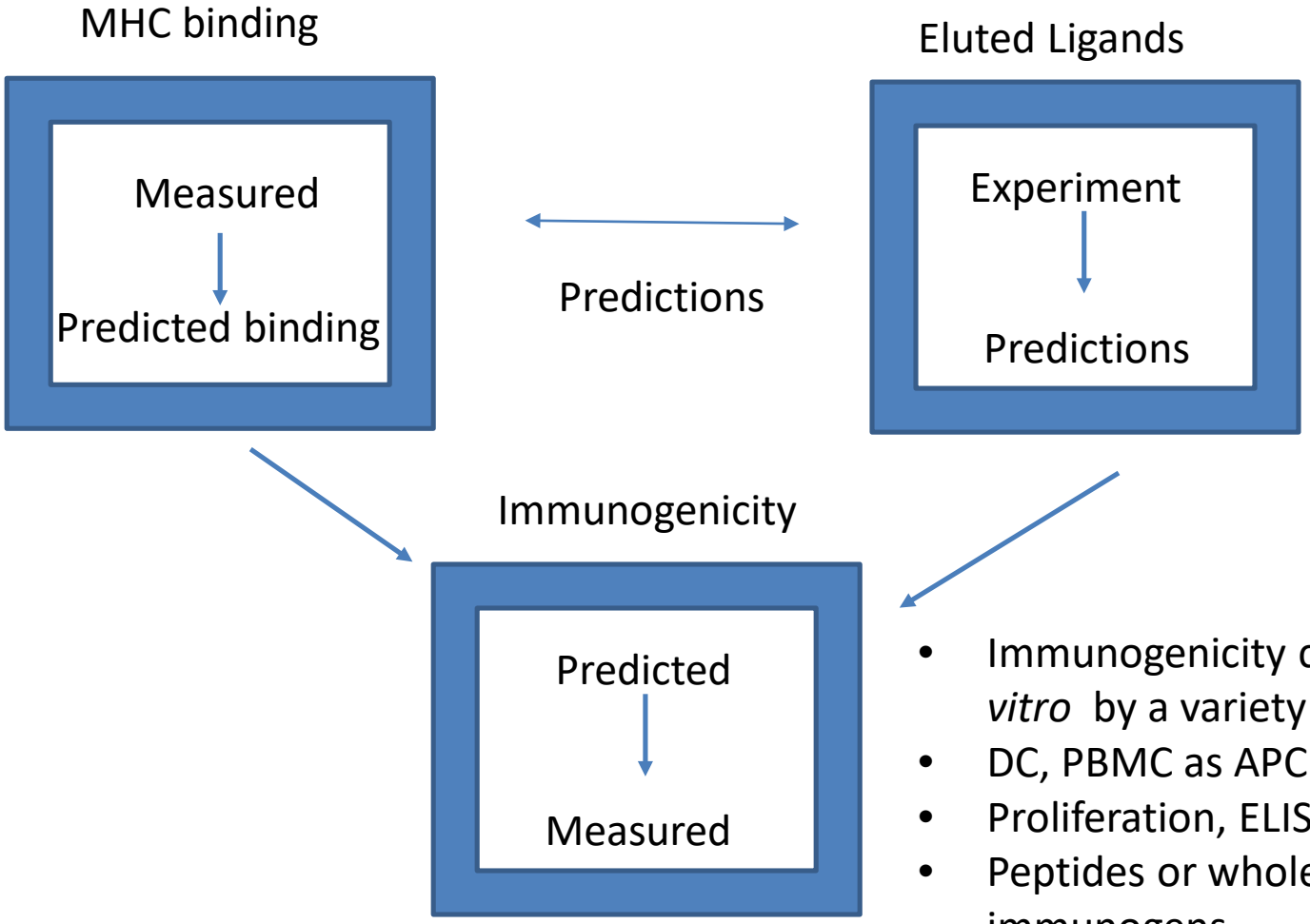
How do algorithms perform in predicting the “other” variable?



Training on eluted data best for predicting eluted ligands
Training on binding data best for predicting binding

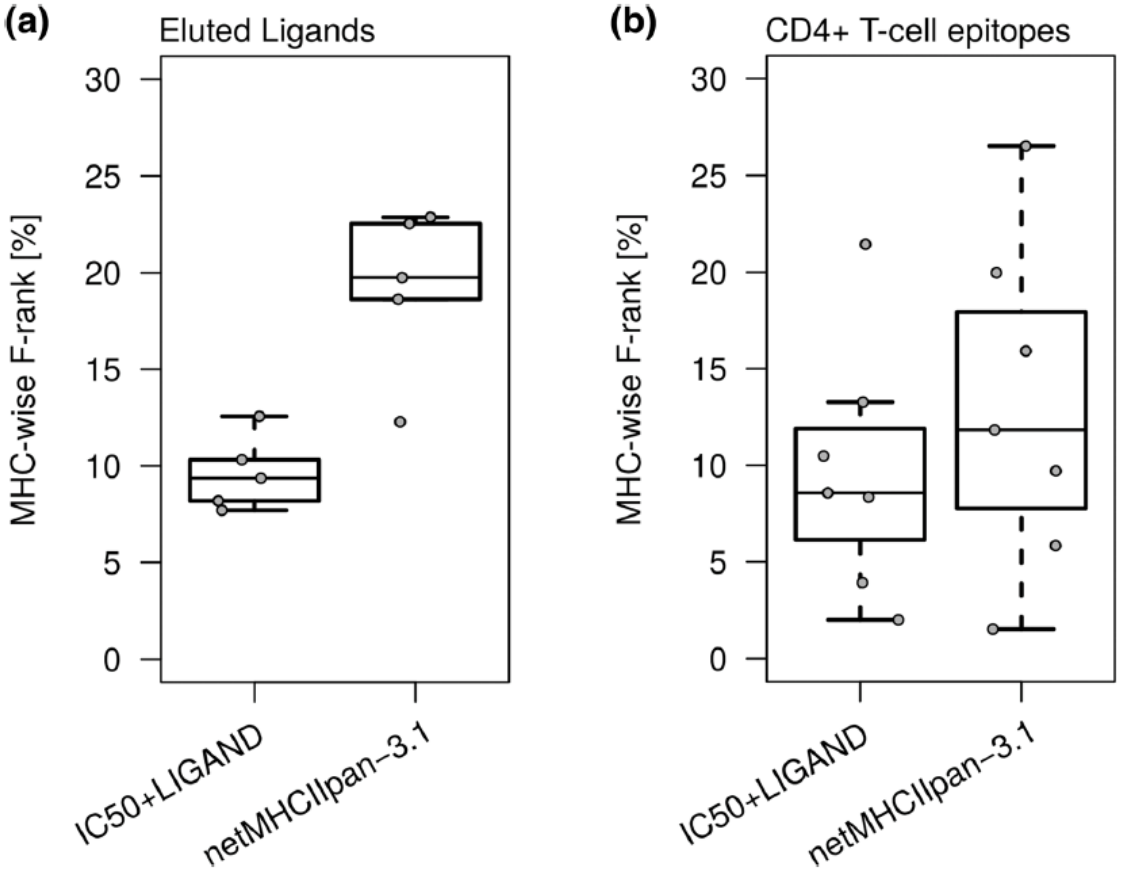
Garde C. Immunogenetics. 2019 Jul;71(7):445-454. PMID: 31183519

What is the relative value of binding versus elution predictions to predict immunogenicity?



- Immunogenicity can be measured *in vitro* by a variety of methods
- DC, PBMC as APC
- Proliferation, ELISPOT, ICS
- Peptides or whole proteins as immunogens

Validation of the approach on external data sets



Garde C. Immunogenetics. 2019 Jul;71(7):445-454. PMID: 31183519

Do T cell responses correlate with ADA?

Measure memory T cell responses in Drug exposed individuals

Do magnitude and/or specificity correlate with ADA titer and/or neutralizing activity?

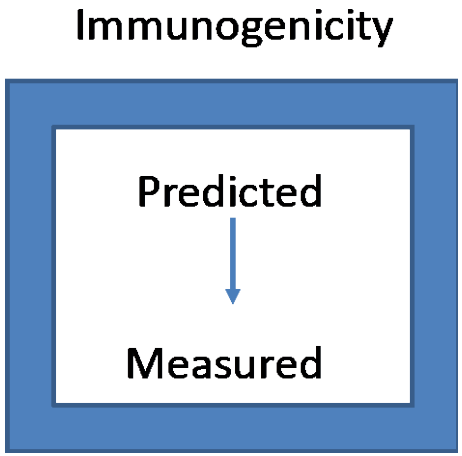
Does immunogenicity (predicted or measured or in non-exposed subjects) predict immunogenicity in exposed subjects?

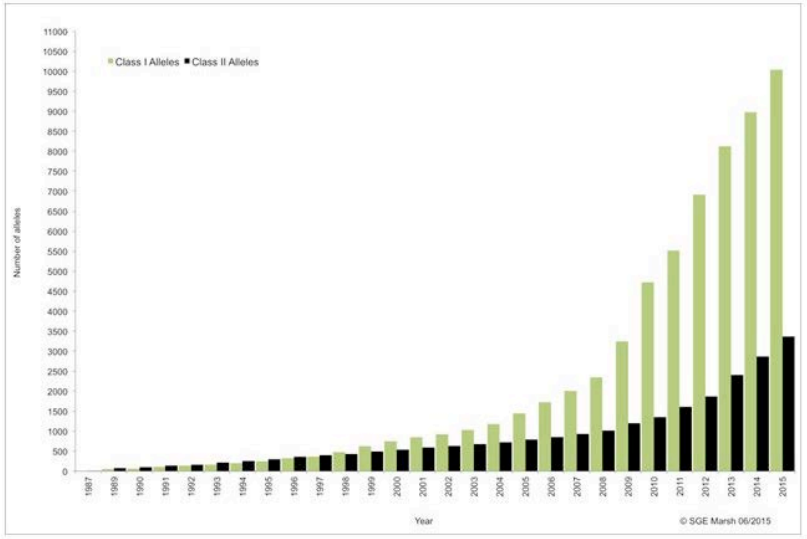
Are the same epitopes recognized as dominant in ADA+ and naïve subjects (implications for de-immunization)?

Conclusions. I

- A variety of tools are available to measure or predict immunogenicity
- MHC binding and eluted ligands are effective as a first step
- Combining methodology offers a limited but significant gain
- The field would benefit from objective benchmarking of how well binding and in vitro immunogenicity predict
  - in vivo immunogenicity
  - ADA

The impact of HLA polymorphism on binding versus immunogenicity predictions





- Thousands of different HLA class II variants exist
- Responding/treated populations express hundreds of alleles
- Individual subjects express up to 8 different variants
- HLA binding predictions are allele specific
- Actionable strategies to target not alleles, but individuals and populations, are required

Two main scenarios need to be considered

- Specific HLA types are associated with immunogenicity issues
- Prediction of HLA–associated immunogenicity in a general population (worldwide or specific ethnicity)

HLA Associations with Autoimmune Diseases

Associations of HLA serotype with susceptibility to autoimmune disease		
Disease	**HLA allele**	**Relative risk**
Ankylosing spondylitis	B27	87.4
Acute anterior uveitis	B27	10.04
Goodpasture's syndrome	DR2	15.9
Multiple sclerosis	DR2	4.8
Graves' disease	DR3	3.7
Myasthenia gravis	DR3	2.5
Systemic lupus erythematosus	DR3	5.8
Insulin-dependent diabetes mellitus	DR3/DR4 Heterozygote	~25
Rheumatoid arthritis	DR4	4.2
Pemphigus vulgaris	DR4	14.4
Hashimoto's thyroiditis	DR5	3.2

Most associations are with Class II; balance between wanted and unwanted reactions

Drugs with HLA Associations

DRUG	Associated HLA
CARBAMAZEPINE	HLA-B*1502
ALLOPURINOL	HLA-B*1801
OXICAM	HLA - A2, -B12
ABACAVIR	HLA-B*5701
AMINOPENICILLINS	HLA-A2, -Drw52
ASPIRIN	HLA-DRB1*1302 HLA-DQB1*6690
LAMOTRIGINE	HLA-B*5801
COTRIMOXAZOLE	HLA-A30, -B13, 1-Cw6
NSAIDS	HLA-DR
FLUCLOXACILLIN	HLA-B*5701
CO-AMOXICLAV	HLA-DRB1*1501
DICLOFENAS	HLA-DRB1*13

Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire
Ostrov et al PNAS 2012

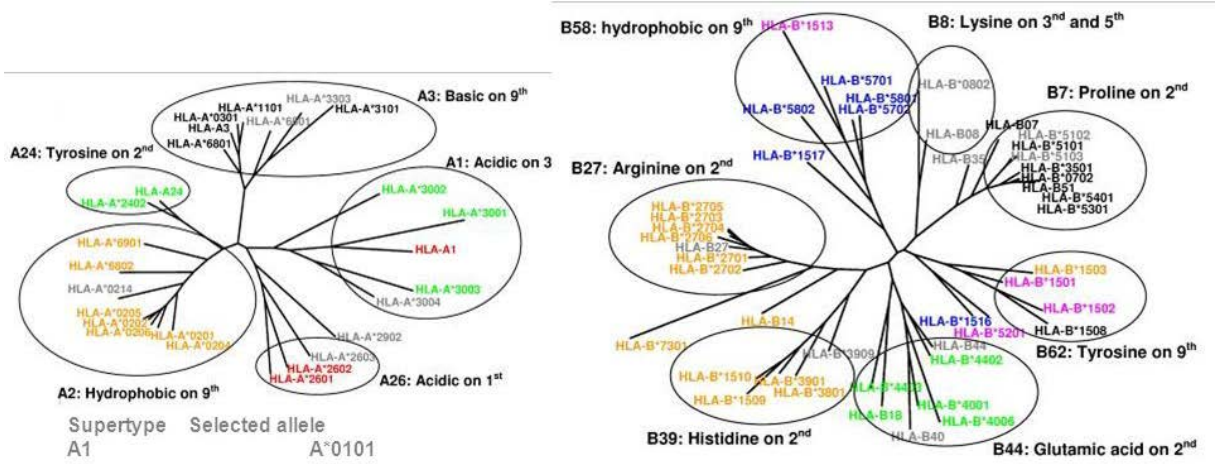
Considerations on predictions for specific HLA alleles

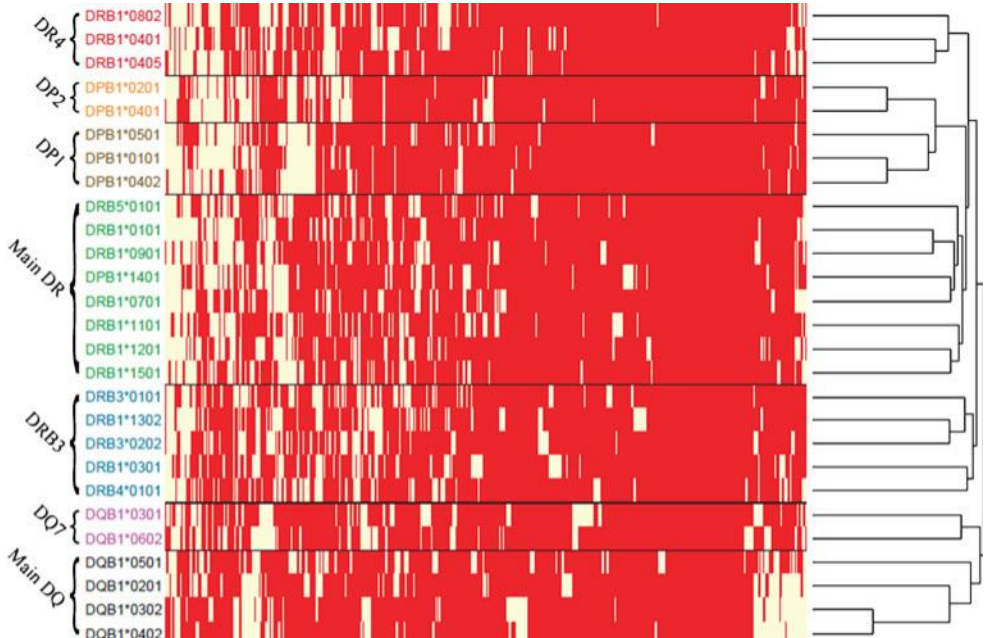
- To the best of my knowledge, ADA has not been associated to specific HLA alleles
- This is not been routinely addressed (by HLA typing ADA+ cases vs ADA- subjects)
- If a specific association was detected, de-immunization and administration strategies could take advantage of the information

Prediction of HLA–associated immunogenicity in a general population (worldwide or specific ethnicity)

- The frequency of different HLA alleles varies dramatically in different ethnicities
- HLA polymorphism results from local genetic pressure generated by pathogens trying to escape HLA recognition
- Evidence in humans (HIV, DENV)

The motifs of the majority of HLAs can be classified in few “Supertypes”





- Several HLA alleles share overlapping peptide specificities (Sidney et al. 1995)
- Seven main HLA class II supertypes and large repertoire sharing across supertypes (Greenbaum et al 2011

The frequency of the different supertypes is conserved in different ethnicities

	Specificity		Phenotypic frequency				
Supertype	Position 2	C-terminus	Caucasian	N.A. Black	Japanese	Chinese	Hispanic
B7	P	AILMVFWY	43.2	55.1	57.1	43.0	49.3
A3	AILMVST	RK	37.5	42.1	45.8	52.7	43.1
A2	AILMVT	AILMVT	45.8	39.0	42.4	45.9	43.0
A24	YF [WIVLMT]	FI [YWLM]	23.9	38.9	58.6	40.1	38.3
B44	E [D]	FWYLIMVA	43.0	21.2	42.9	39.1	39.0
A1	TI [LVMS]	FWY	47.1	16.1	21.8	14.7	26.3
B27	RHK	FYL [WMI]	28.4	26.1	13.3	13.9	35.3

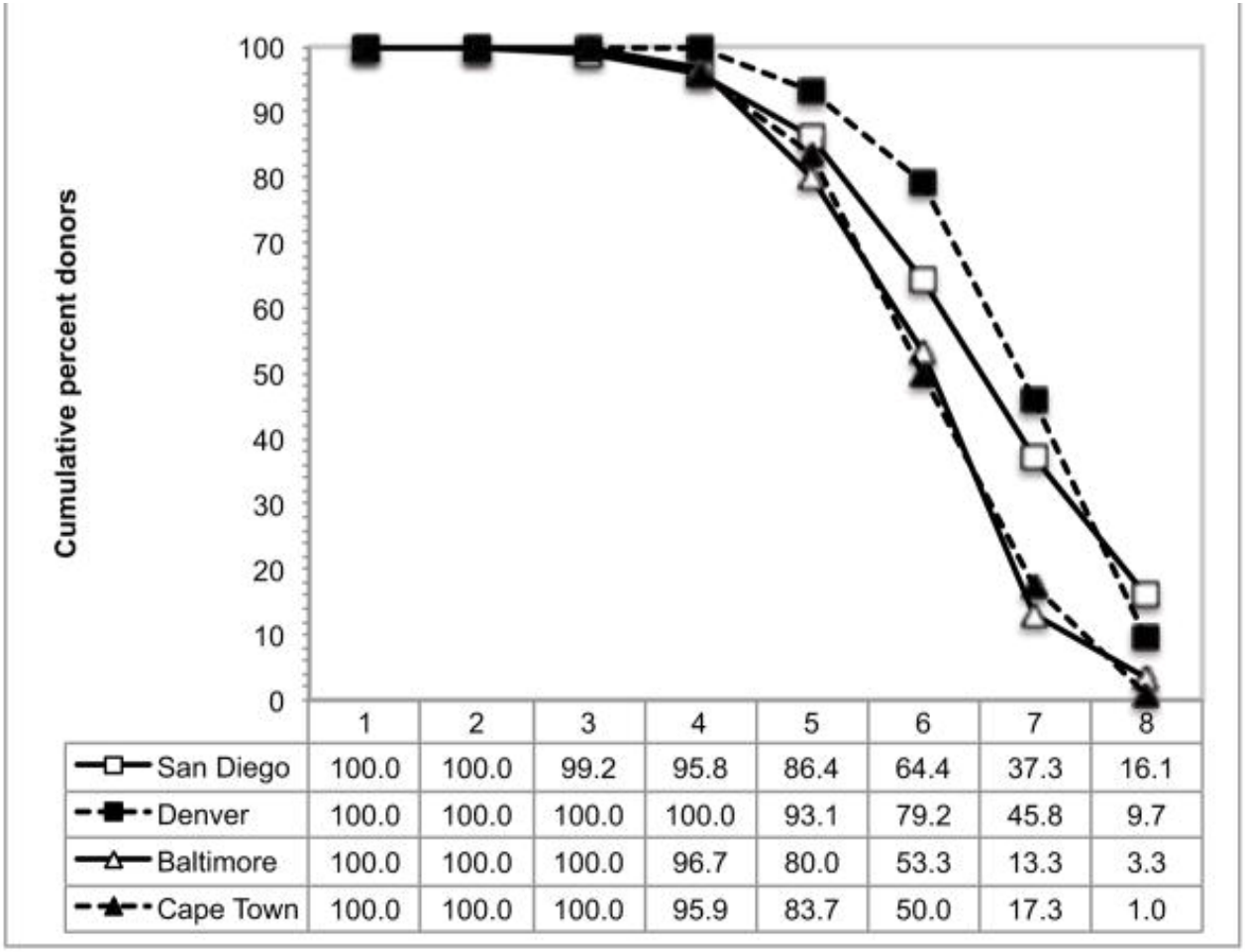
26 HLA class II alleles allow for representative global coverage

Locus	Molecule	Phenotype frequency
DRB1	DRB1*0101	5.4
	DRB1*0301	13.7
	DRB1*0401	4.6
	DRB1*0405	6.2
	DRB1*0701	13.5
	DRB1*0802	4.9
	DRB1*0901	6.2
	DRB1*1101	11.8
	DRB1*1201	3.9
	DRB1*1302	7.7
	DRB1*1501	12.2
	Combined	71.1
DRB3/4/5	DRB3*0101	26.1
	DRB3*0202	34.3
	DRB4*0101	41.8
	DRB5*0101	16.0
	Combined	87.7

Locus	Molecule	Phenotype frequency
DQA1/DQB1	DQA1*0501/DQB1*0201	11.3
	DQA1*0501/DQB1*0301	35.1
	DQA1*0301/DQB1*0302	19.0
	DQA1*0401/DQB1*0402	12.8
	DQA1*0101/DQB1*0501	14.6
	DQA1*0102/DQB1*0602	14.6
	Combined	81.6
DPA1/DPB1	DPA1*0201/DPB1*0101	16.0
	DPA1*0103/DPB1*0201	17.5
	DPA1*01/DPB1*0401	36.2
	DPA1*0301/DPB1*0402	41.6
	DPA1*0201/DPB1*0501	21.7
	DPB1*1401@	7.4
	Combined	94.5

McKinney et al Immunogenetics. 2013 May; 65(5): 357–370.

HLA coverage in different ethnicities



	1	2	3	4	5	6	7	8
San Diego	100.0	100.0	99.2	95.8	86.4	64.4	37.3	16.1
Denver	100.0	100.0	100.0	100.0	93.1	79.2	45.8	9.7
Baltimore	100.0	100.0	100.0	96.7	80.0	53.3	13.3	3.3
Cape Town	100.0	100.0	100.0	95.9	83.7	50.0	17.3	1.0

McKinney et al [Immunogenetics. 2013 May; 65(5): 357–370.]

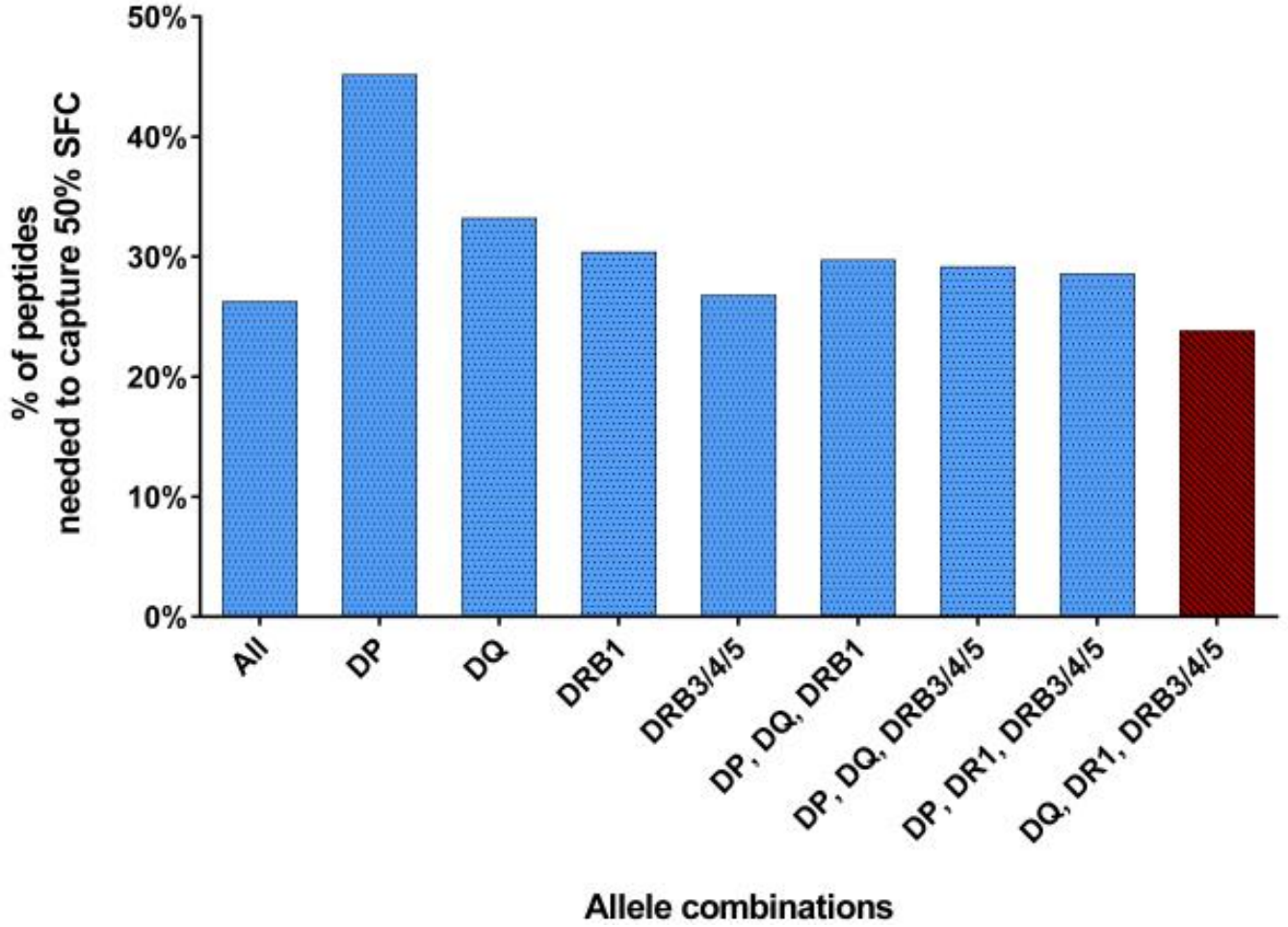
Optimization to predict dominant epitopes

- Peptide datasets spanning entire proteins associated with measured immune responses in exposed humans

Dataset	No. of Antigens	Total peptides	No. of donors	Reference
Der p/f (House dust mite)	4	156	20	Hinz,2015
Phl p (Timothy grass)	10	425	25	Oseroff, 2010
TB-1	4	71	18	Arlehamn, 2012
TB-2	11	499	32	Arlehamn,2015
Cockroach	6	463	19	Oseroff, 2012
Pertussis	9	785	23	Dillon, 2015
TOTAL	44	2399	137	

Paul S et al. Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes. J Immunol Methods. 2015 PubMed PMID: 25862607

Optimizing predictions by an iterative agnostic process



Key outcomes of the Paul et al. study

- Empirically derived a set of 7 alleles as being optimal
- Seven DRB alleles (DRB1*03:01,*07:01,*15:01, DRB3*01:01,*02:02, DRB4*01:01, DRB5*01:01) associated with optimal performance
- DR alleles are the most dominant locus restricting HLA class II responses in humans.
- The seven allelic variants cover the previously described main HLA class II supertypes

Further validation in predicting immunogenicity in human populations

- Considered an agnostic approach, were we used T cell epitope data to directly train predictive algorithms
- Used in-house data and IEDB-derived tetramer as training set
- 57 studies from other labs using overlapping peptides and exposed populations contained 530 non-redundant dominant epitopes and 1758 non-epitopes as validation set

Predicting HLA CD4 Immunogenicity in Human Populations.Dhanda , Karosiene , Edwards , Grifoni , Paul , Andreatta , Weiskopf , Sidney , Nielsen, Peters and Sette. Front Immunol. 2018 Jun 14;9:1369. PMID: 29963059

Conclusions. II

- Allele specific ADA effects could be targeted by allele-specific strategies
- Global predictions on a general population level
  - Strategies have been developed and validated based on human subjects data