M1012

Predicting Immunogenicity in the Era of Personalized Medicine: A Need for Individual Risk Assessment

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ABSTRACT

Purpose: While immunogenicity of biologics is widely acknowledged as a barrier to clinical success, the means for pre-screening patients and identifying those at risk of developing anti-drug antibodies (ADA) have been lacking. Several studies have identified HLA as one potential biomarker of immunogenicity, leading to the concept that immunogenicity can be screened using computational and experimental methods that define HLA-binding peptides at pre-clinical and clinical stages of development. Here we report the use of our "Individualized T Cell Epitope Measure" (iTEM) tool for analyzing the HLA-dependent immunogenicity of 23 monoclonal antibodies in clinical use and its validation using clinical observations from antibody-treated patients.

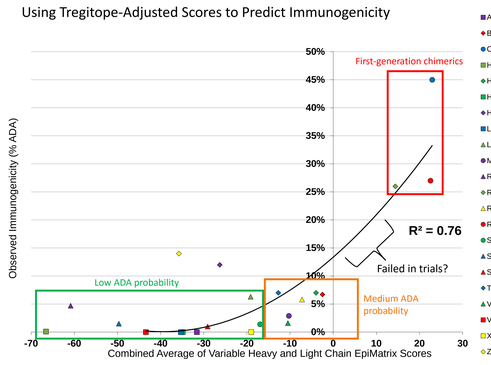
Methods: The iTEM tool, a component of EpiVax's ISPRI platform and used for screening biologic sequences, examines protein sequences for potential immunogenicity based on an individual subject's HLA phenotype. Each of the input protein's 9-mer frames is analyzed for potential binding to the individual's HLA using the EpiMatrix system, resulting in a patient-specific iTEM score. A positive (or negative) iTEM score corresponds to an increased (or decreased) T cell epitope content, compared to a random protein of similar length. Pairs of high-, moderate-, and low-risk HLA haplotypes can be identified for each of the monoclonal antibodies. The same approach was applied to data available from cohorts of patients suffering from various autoimmune disorders: Psoriatic Arthritis (PsA), Ankylosing Spondylitis (AS), and Rheumatoid Arthritis (RA). These patients were exposed to adalimumab and both HLA phenotypes and anti-adalimumab antibody titers detected at various time points were available.

Results: Heat maps were developed for 23 licensed monoclonal antibodies, revealing pairs of HLA high-risk alleles that may be linked to higher ADA titers in selected patients. For example, in adalimumab, HLA-DR1 and HLA-DR7 are associated with higher putative T cell epitope content, compared to other HLA-DR alleles, which may contribute to higher levels of ADA in subjects expressing those alleles. Conversely, in natalizumab, pairs of HLAs comprising HLA-DR15 are associated with lower putative T cell epitope content, suggesting that HLA-DR15 may decrease the risk of ADA formation in patients expressing this allele. For a retrospective set of study patients, potential risk for ADA to adalimumab was determined using the iTEM tool on each cohort separately. ADA responses were higher in PsA and AS patients whose iTEM score was elevated compared to low-risk patients. No associations between ADA responses and iTEM scores could be made with the RA cohort. We hypothesize that ADA responses in these patients highly depend on the functionality of their regulatory T cells.

Conclusions: We have analyzed the immunogenicity of monoclonal antibodies and identified at-risk combinations of HLA haplotypes. We also found that our HLA-specific immunogenicity predictions using the iTEM tool correlated with observed immunogenicity, representing an important step forward in personalized medicine.

INDIVIDUALIZED T CELL EPITOPE MEASURE (iTEM): PERSONALIZED IMMUNOGENICITY ASSESSMENT

Using Tregitope-Adjusted Scores to Predict Immunogenicity



- Immunogenicity is predicted for a set of eight common HLA Class II alleles, covering over 95% of the global human population.*
- EpiMatrix predicts immunogenicity of mAb sequences as the sum of T effector epitopes, adjusted for Treg epitope (Tregitope) content.**
- Predicted immunogenicity adjusted for Tregitope content correlates with observed immunogenicity.***

* Southwood, S et al. Several common HLA-DR types share largely overlapping peptide binding repertoires. *Journal of Immunology*. (1998) 160:3363-3373

** Weber, CA et al. T cell epitope: friend or foe? Immunogenicity of biologics in context. *Adv Drug Deliv Rev.* (2009) 61(11):965-76

*** De Groot, AS & Martin, W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. *Clinical Immunol*. (2009) 131(2):189-201

Monoclonal Antibody iTEM Score:

0 + 1 - regulatory T cell epitope = response

T cell response depends on:
HLA of subject + T effector epitope content + Tregitope content

EpiMatrix Protein Report

File:Your File - Sequence: Your Protein

Frame Start	AA Sequence	Frame Stop	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
1	PCPAPELLG	9	-1.02	-0.43	-0.47	-1.1	-0.36	0.55	-0.29	-0.26	0
2	CPAPELLGG	10	-2.13	-1.25	-1.28	-2.54	-1.07	-1.1	-0.42	-0.68	0
55	VEVHNAKTK	63	0.26	-0.63	0.54	1.23	-0.04	0	1.11	0.06	0
56	EVHNAKTKP	64	-0.39	0.44	-0.29	-0.95	0.64	0.27	0.44	-0.47	0
57	VHNAKTKPR	65	1.74	2.1	1.85	1.7	2.06	1.18	2.25	1.59	6
58	HNAKTKPRE	66	-1.27	0.63	-1.59	-1.64	2.1	0.52	1.17	-0.9	1
59	NAKTKPREE	67	-0.55	-0.74	-0.8	-0.24	0.2	-0.78	-0.37	-1.21	0
68	QYNSTYRVV	76	0.55	-0.52	-0.74	0.9	-1.44	-0.09	-1.3	-0.41	0
69	YNSTYRVVS	77	1.9	1.42	1.22	1.38	2.26	1.41	2.2	1.41	3
70	NSTYRVVSV	78	-0.46	-0.95	-0.34	0.3	-0.29	-1.09	0.38	-0.52	0
71	STYRVVSVL	79	0.34	-0.34	-0.47	0.67	0	-0.46	0.63	-0.18	0
72	TYRVVSVLT	80	1.49	-0.11	1.19	1.66	0.5	0.56	0.09	1.19	1
73	YRVVSVLTV	81	1.94	1.24	2.03	2.63	1.05	2	1.57	1.31	4
74	RVVSVLTVL	82	-0.32	1.09	-0.79	0.21	-0.64	-0.82	0.74	0.74	0
75	VVSVLTVLH	83	1.68	1.85	2.42	1.38	2.1	1.67	2.02	1.52	6
76	VSVLTVLHQ	84	1.01	0.5	1.82	1.04	1.1	1.66	0.86	0.54	2
77	SVLTVLHQD	85	-0.93	-0.69	-1.07	-0.14	0.26	-1.18	0.17	-0.52	0
78	VLTVLHQDW	86	-0.06	0.81	-0.31	1.18	-0.07	0.15	-0.2	1.05	0
207	NHYTQKSLS	215	0.42	0.73	0.27	-0.09	1.24	0.87	1.43	0.93	0
208	HYTQKSLSL	216	1.44	0.63	1.24	1.46	0.52	0.94	1.49	1.46	0
209	YTQKSLSLS	217	0.68	1.68	0.76	0.86	2.46	2.02	2	0.94	4
210	TQKSLSLSP	218	0.8	0.75	1.4	1.54	0.23	1.09	0.56	0.8	0
211	QKSLSLSPG	219	0.68	0.54	0.67	-0.18	1.54	1.42	0.65	0.95	0
212	KSLSLSPGK	220	0.66	0.57	0.94	0.39	0.47	1.02	0.33	0.8	0

Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-score	2.18	2.5	2.42	2.63	2.47	2.41	2.84	2.49	--
Sum of Significant Z-scores	20.14	23.2	22.19	26.64	27.15	20.78	21.88	10.08	172.05
Count of Significant Z-Scores	11	12	11						
Total Assessments Performed: 1696			Deviation from Expect				Deviation		
Adjusted for Regulatory Epitopes			Deviation from Expect				Deviation		

High T cell epitope content

Low T cell epitope content

Z-score indicates the potential of a 9-mer frame to bind to a given HLA allele. All scores in the Top 5% (Z-Score ≥ 1.64) are considered "Hits".

The strength of the score is indicated by the differences in blue shading. For example: Top 10% Top 5% Top 1%

Frames containing four or more alleles scoring above 1.64 are referred to as Epi-Bars and are highlighted in yellow.

Frames conserved in IgG antibodies and believed to be either passively tolerated or actively regulatory are highlighted in green.

- Epitope content is dependent on HLA binding potential. Different alleles have different affinities to the same peptides.
- The iTEM tool estimates a protein's immunogenicity by analyzing its T cell epitope content from an individual's HLA perspective. This results in a patient-specific iTEM score.
- iTEM scores can be summarized as heat maps, revealing pairs of high- and low-risk HLA haplotypes.

iTEM Heat map

Sequence: Your Protein

HLA freq.*	DRB1*0101 10.4	DRB1*0301 11.9	DRB1*0401 24.4	DRB1*0701 14	DRB1*0801 15.1	DRB1*1101 15.5	DRB1*1301 15.1	DRB1*1501 20.5
DRB1*0101		--	--	--	--	--	--	--
DRB1*0301			--	--	--	--	--	--
DRB1*0401				--	--	--	--	--
DRB1*0701					--	--	--	--
DRB1*0801						--	--	--
DRB1*1101							--	--
DRB1*1301								--
DRB1*1501								

iTEM evaluation for a DR11 / DR13 patient

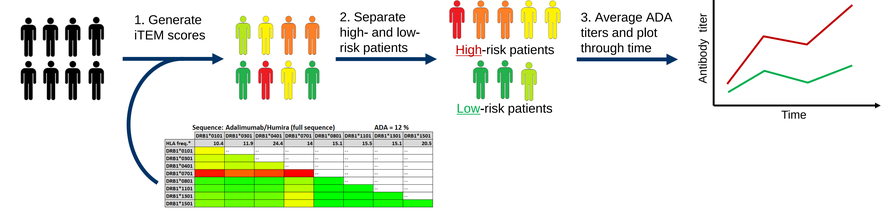
Predicted risk of ADA formation: Low — Medium — High

CASE STUDY: iTEM ANALYSIS OF PATIENTS EXPOSED TO ADALIMUMAB

We retrospectively analyzed three cohorts of patients exposed to adalimumab and for which both ADA responses and HLA types were known.

Hypothesis: At-risk patients (with an elevated iTEM score) will develop a stronger ADA response to adalimumab than low-risk patients.

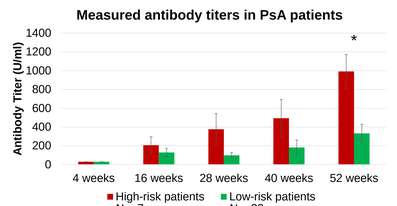
Methods:



Results:

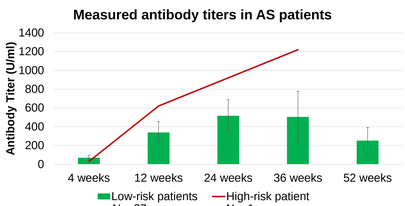
Cohort 1: 40 Psoriatic Arthritis (PsA) patients.

- **High-risk patients developed higher antibody titers.**



Cohort 2: 28 Ankylosing Spondylitis (AS) patients.

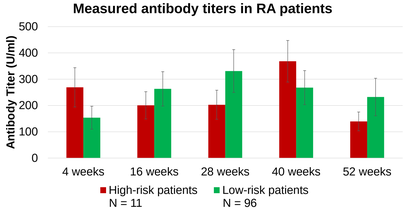
- **High-risk patient developed higher antibody titers.**



Cohort 3: 107 Rheumatoid Arthritis (RA) patients.

- Inconclusive results.
- Many studies have shown that RA patients do not have fully functional Tregs.*
- **Treg functionality may be a key factor to explain ADA responses.**

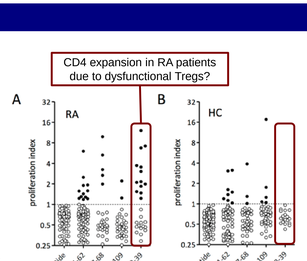
*Ehrenstein MR et al., Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFα therapy. J Exp Med. (2004) 200:3:277-285
Cribbs AP et al., Treg cell function in rheumatoid arthritis is compromised by CTLA-4 promoter methylation resulting in a failure to activate the indoleamine 2,3-dioxygenase pathway, Arthritis Rheumatol. (2014) 66:9:2344-2354



iTEM ANALYSIS OF RHEUMATOID ARTHRITIS PATIENTS: ADJUSTING FOR TREG FUNCTIONALITY?

- Overall CD4 T cell proliferation after 14 days in PBMC cultured against different adalimumab-derived peptides in RA patients (A) and Healthy Control (HC) donors (B).
- L18-39, **a Treg epitope-containing peptide from adalimumab**, induced CD4 T cells proliferation in some RA patients but not in HC.

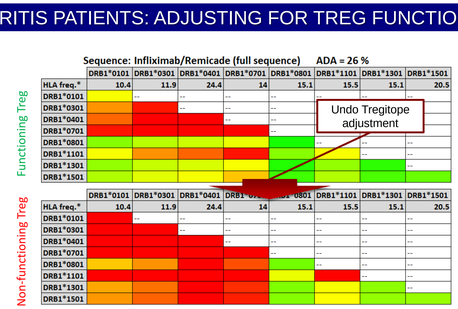
M. Makuch, J. van Beek, C.A. Wijbrandts, J. van der Griendt, M. Aalbers, P. Stas, A.B. Meijer, A. ten Brinke, T. Rispens, P.P. Tak, G.J. Wolbink, J. Schuurman, P.W.H.I. Parren, S. M. van Ham. The TNFα-binding domain of the therapeutic antibody adalimumab elicits CD4 T-cell responses in Rheumatoid Arthritis patients. (Manuscript in preparation)

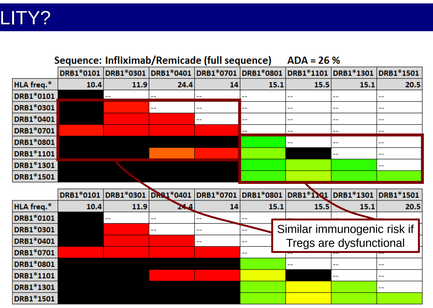


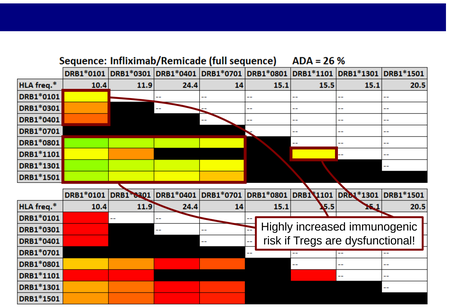
- A recent study showed that **~50% of RA patients** developed an ADA response to infliximab and its biosimilar.* The same therapeutics induced an ADA response in **~20% of AS patients**.**
- Is the increased ADA response in RA patients due to a lack of Treg functionality?
- **iTEM can be adjusted to take into consideration Treg functionality, if known.**

* Yoo DH et al., A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: the PLANETRA study.. Ann Rheum Dis. (2013) 72:1613-1620

** Park, W et al. Comparable long-term efficacy, as assessed by patient-reported outcomes, safety and pharmacokinetics, of CT-P13 and reference infliximab in patients with ankylosing spondylitis : 54-week results from the randomized, parallel-group PLANETAS study. Arthritis Research & Therapy. (2016) 18:25







CONCLUSIONS

- ✓ The individualized T cell Epitope Measure (iTEM) tool tailors immunogenicity predictions to a patient's specific HLA phenotype.
- ✓ iTEM allows the identification of HLA haplotypes that are associated with a greater risk of developing anti-drug antibody (ADA) responses.
- ✓ In a retrospective analysis, we identified patients within three cohorts that were at risk of developing an ADA response to adalimumab based on their iTEM scores. Higher antibody titers against adalimumab were observed in the PsA and AS while no association could be observed in the RA cohort.
- ✓ Other studies have showed increased ADA responses in RA cohorts. **Analyzing Treg functionality in RA cohorts may be critical to understand the development of ADA responses to mAbs.**
- ✓ The iTEM tool can identify at-risk patients of developing ADA responses to therapeutics. This represents an important step forward in personalized medicine.