Enhancing Immunogenicity Risk Assessment: Leveraging ISPRI for Bispecific Antibody Analysis

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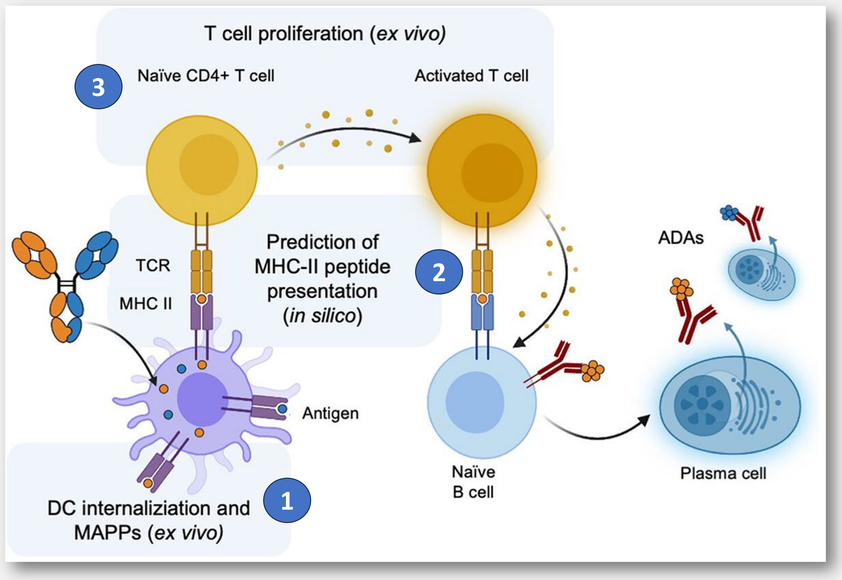
Science without fear

INTRODUCTION

Bispecific antibodies are recombinant molecules designed to target two different epitopes simultaneously. Several engineering techniques have been developed to facilitate their production, including knob-into-holes Fc and Fab mutations. Wen-Ting *et al.* performed an analysis on a panel of bispecific antibodies to assess the immunogenic risk imposed by both types of mutations. They employed both computational and experimental methods to model various steps in the cellular pathway leading to the production of anti-drug antibodies (ADA), including a (1) DC internalization assay to capture the uptake of the protein therapeutic by antigen presenting cells, both an (2) *in silico* prediction tool (NetMHCIIpan-4.0) and an MHC-associated peptide proteomics (MAPPs) assay to model the presentation of the processed peptides by MHC-II, and (3) T cell assays to assess the activation and proliferation of naïve T cells. Based on the results, they assigned an immunogenicity risk to each bispecific antibody and monospecific counterpart.

OBJECTIVE: To perform a retrospective comparative analysis to assess the same panel of bispecific antibodies using in silico prediction tools hosted on ISPRI (Interactive Screening and Protein Reengineering Interface) and compare results to published data from Wen-Ting *et al.* as well as available clinical data.

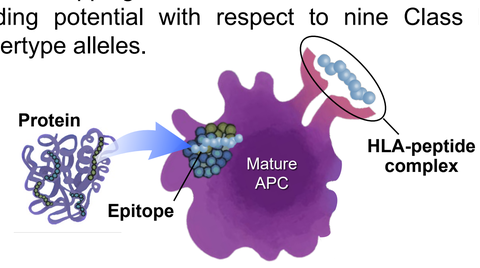


METHODS

Performed an immunogenicity analysis using in silico prediction tools hosted on ISPRI (Interactive Screening and Protein Reengineering Interface)

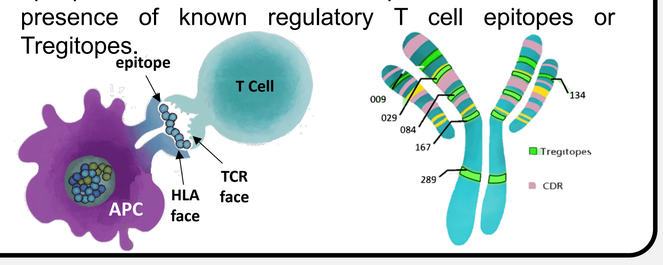
1 EpiMatrix

EpiMatrix™ parsed each bispecific antibody sequence into overlapping 9-mer frames and assessed for HLA binding potential with respect to nine Class II HLA supertype alleles.



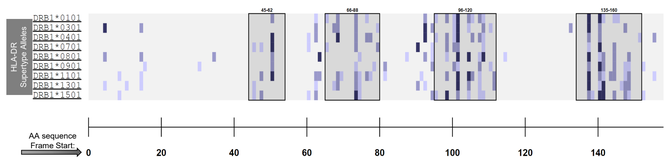
2 JanusMatrix and Tregitope-Adjustment

JanusMatrix™ searched for potentially tolerated epitopes based on shared TCR-facing contours with epitopes derived from the human proteome and for the presence of known regulatory T cell epitopes or Tregitopes.



3 ClustiMer

ClustiMer™ identified T cell epitope dense regions referred to as clusters. Generated epitope maps to provide a visual of all epitope content including clusters across the whole bispecific sequence.



4 Immunogenic Peptide Counts

Based on a review of the EpiMatrix detailed reports, an immunogenic peptide count was compiled for each bispecific antibody and monospecific counterpart.

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*0901 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits	AbDB[1] Frequency (%)	Germline[2] Count
1	DIQMTQSPSS	9	0.91	0.17	0.26	0.08	0.09	1.42	0.86	-0.24	-0.04	0	20.35	37
2	IQMTQSPSSL	10	2.15	1.35	2.26	1.58	2.17	1.40	1.88	2.24	2.52	6	15.97	33
3	QMTQSPSSLS	11	1.11	0.28	0.50	0.96	0.29	0.32	0.39	0.90	0.40	0	14.94	29
4	MTQSPSSLSA	12	1.87	2.26	2.02	1.88	1.06	2.31	1.66	1.79	2.05	6	23.95	29
5	TQSPSSLSAS	13	0.51	0.28	1.31	0.69	-0.04	0.47	0.54	1.09	1.05	0	30.07	36
▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪
46	LLIYAASTL	54	1.41	1.08	0.99	2.16	1.54	2.30	0.85	2.28	2.30	4	3.73	6
47	LIYAASTLQ	55	1.91	1.85	2.46	2.07	1.79	2.36	1.08	1.79	2.10	8	3.99	6
48	IYAASTLQS	56	1.73	2.01	2.68	1.26	1.58	1.93	1.82	2.11	2.07	7	3.52	6
49	YAASTLQSG	57	0.64	1.60	0.59	0.32	1.42	1.34	1.28	0.04	1.09	0	3.61	6
▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪	▪
205	VTKSFNRGE	213	0.89	1.94	0.88	0.95	1.26	1.92	1.21	1.12	0.60	2	0.00	5
206	TKSFNRGEC	214	0.17	-0.73	-0.79	0.17	0.91	0.21	0.44	-0.58	1.07	0	0.00	5

Summarized Results	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*0901	DRB1*1101	DRB1*1301	DRB1*1501	Total
Maximum Single Z-scores	2.40	2.26	3.16	3.11	2.41	2.71	2.29	2.52	2.52	--
Sum of Significant Z-scores	37.33	26.05	31.83	30.59	14.18	36.40	22.64	22.72	26.87	248.61
Count of Significant Z-scores	19	13	14	15	7	18	12	11	13	122
Total Assessments Performed: 1854	Deviation from Expectation: 42.39							Deviation per 1000 AA: 22.86		
Adjusted for Regulatory Epitopes	Deviation from Expectation: -34.63							Deviation per 1000 AA: -19.68		

5 Immunogenicity Risk Assessment

Based on the collective results from EpiMatrix, JanusMatrix, and ClustiMer, an immunogenicity risk was assigned to each bispecific antibody and monospecific counterpart.

ISPRI is an **integrated** and **secure** set of in silico tools providing a **fast** and **reliable** immunogenicity assessment.

Wen-Ting *et al.* Results

Molecule name	Antibody 1 Identify	Antibody 1 Fc and Fab mutations	Antibody 2 Identity	Antibody 2 Fc and Fab mutations	# host cells in production	Antibody grade
Avastin	Bevacizumab	–	n/a	n/a	1	P
Bevacizumab	Bevacizumab	–	n/a	n/a	1	R
Herceptin	Trastuzumab	–	n/a	n/a	1	P
Trastuzumab	Trastuzumab	–	n/a	n/a	1	R
Tras KIH 1 cell	Trastuzumab	Knob	Trastuzumab	Hole	1	R
Tras KIH 2 cell	Trastuzumab	Knob	Trastuzumab	Hole	2	R
Bev KIH 1 cell	Bevacizumab	Knob	Bevacizumab	Hole	1	R
Bev KIH 2 cell	Bevacizumab	Knob	Bevacizumab	Hole	2	R
Tras/Bev 1 cell v1	Trastuzumab	Knob, EKKE	Bevacizumab	Hole, KEEK	1	R
Tras/Bev 1 cell v2	Trastuzumab	Knob, KEEK	Bevacizumab	Hole, EKKE	1	R
Tras/Bev 2 cell	Trastuzumab	Knob	Bevacizumab	Hole	2	R
Boco/Bev	Bococizumab	Knob	Bevacizumab	Hole	2	R
ATR-107/Bev	ATR-107	Knob	Bevacizumab	Hole	2	R

Table. 1 Panel of Bispecific Antibodies

Low ADA benchmarks: Bevacizumab & Trastuzumab

High ADA benchmarks: Bococizumab & ATR-107

Fig. 2 DC Internalization Results

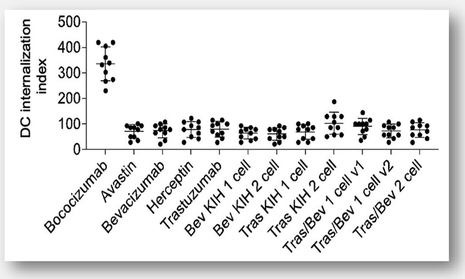
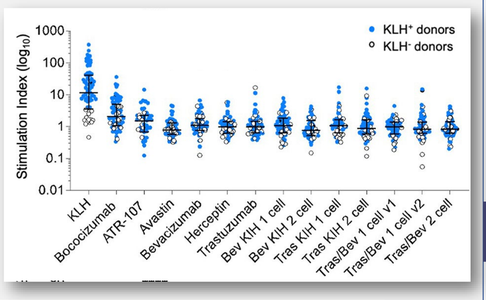


Fig. 3 T Cell Proliferation Results



All bispecific antibodies perform similarly to low ADA benchmarks for both the DC internalization and T cell proliferation assays.

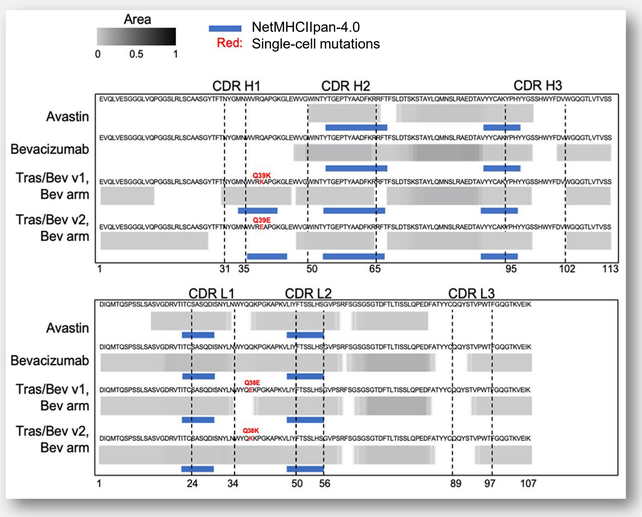


Fig. 4 MAPPs and in silico Results

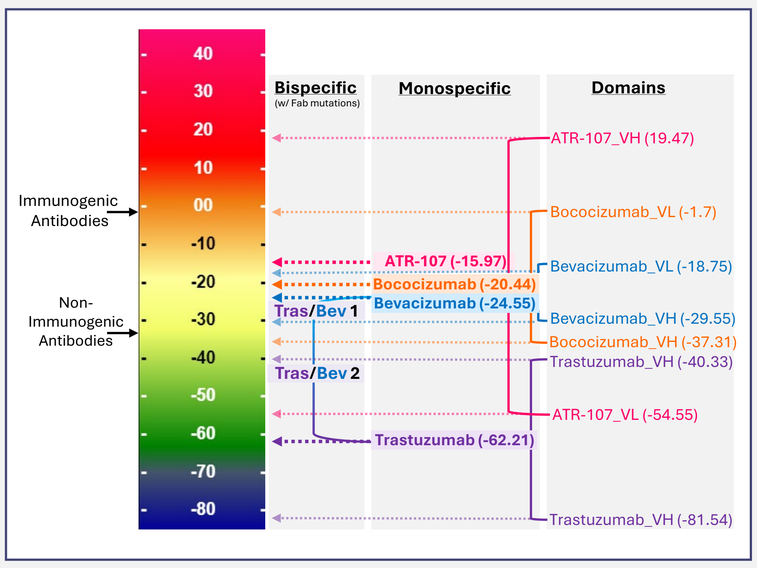
Gray bars: MAPPs peptide spectrum matches

Blue bars: ***P***eptides predicted to bind at least two HLA-DR alleles excluding epitopes highly conserved in the human proteome.

ISPRI Results

Input Data: Molecule Name	Arm	Domain	Domain Analysis: EPX Score	Domain Analysis: JMX Score	Paired Analysis: EPX Score	Paired Analysis: JMX Score	Paired Analysis: Medium & High Risk Clusters
Bevacizumab	Bevacizumab	VH	-29.55	13.81	-24.55	12.48	3
		VL	-18.75	11.04			
Trastuzumab	Trastuzumab	VH	-81.54	21.35	-62.21	15.67	1
		VL	-40.33	11.18			
Tras/Bev 1 cell v1	Bevacizumab	VH	-4.43	11.12	-27.20	12.31	4
		VL	-8.11	10.47			
	Trastuzumab	VH	-65.47	19.38			2
		VL	-29.45	10.31			
Tras/Bev 1 cell v2	Bevacizumab	VH	-13.94	12.38	-26.18	12.24	4
		VL	-2.79	9.97			
	Trastuzumab	VH	-54.67	16.88			2
		VL	-31.56	10.70			
Tras/Bev 2 cell	Bevacizumab	VH	-29.55	13.81	-43.25	13.88	3
		VL	-18.75	11.04			
	Trastuzumab	VH	-81.54	21.35			1
		VL	-40.33	11.18			
ATR-107	ATR-107	VH	19.47	8.25	-15.97	6.62	5
		VL	-54.55	2.39			
Bococizumab	Bococizumab	VH	-37.31	6.32	-20.44	7.02	4
		VL	-1.70	7.51			

EPX Score: EpiMatrix Score (adjusted for the presence of Tregitopes)
Font color indicates immunogenic potential; **red** for significant, **orange** for moderate, **green** for low, and **blue** for minimal immunogenic potential.
JMX Score: JanusMatrix Score
Green shading indicates potential for homology-induced tolerance; dark green for significant and light green for elevated potential for homology-induced tolerance.



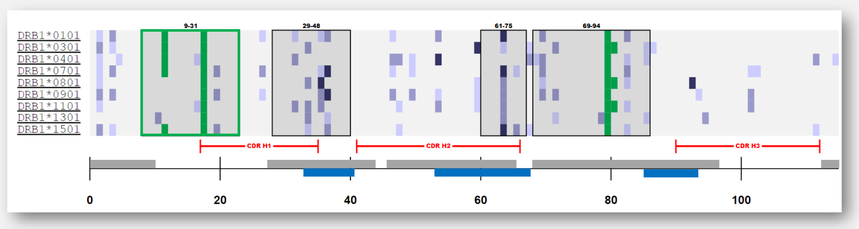
Wen-Ting *et al.* and ISPRI Integrated Results

Overlayed Wen-Ting *et al.* MAPPs and in silico figures (Fig. 4) with epitope maps generated using ISPRI. Tras-Bev 1 Cell V1 bispecific antibody figure is shown on the right as an example.

Discounted due to 'humanness'
NetMHCIIpan-4.0*
MAPPs

*filtered by Observed Antibody Space (OAS) & PEPMatch to exclude epitopes highly conserved in the human proteome.

Tras-Bev 1 Cell V1 Hole-VH



The strength of the score is indicated by the differences in blue shading: Top 10% | Top 5% | Top 1%
Green shading indicates the presence of a Tregitope.

Immunogenic Peptide Counts

Molecule Name	Predicted Immunogenic T Cell Epitopes: NetMHCIIpan-4.0	Overlap with MAPPs	EpiMatrix	Overlap with MAPPs
Bevacizumab	7	6	14	14
Trastuzumab	10	3	9	7
Tras/Bev 1 cell v1	28	16	31	30
Tras/Bev 1 cell v2	26	12	31	26
Tras/Bev 2 cell	19	9	23	21
ATR-107	11	9	8	8
Bococizumab	11	11	10	10

Immunogenicity Risk Assessment

Molecule Name	DC Internalization	T Cell Proliferation	MAPPS& NetMHCIIpan-4.0	Combined Immunogenicity Prediction	Clinical ADAs	ISPRI
Bevacizumab	Low	High	Low	Intermediate	<1%	Low
Trastuzumab	Low	Low	Low	Low	<1%	Low
Tras/Bev 1 cell v1	Low	Low	High	Intermediate	N/A	Intermediate
Tras/Bev 1 cell v2	Low	Low	High	Intermediate	N/A	Intermediate
Tras/Bev 2 cell	Low	Low	Intermediate	Low	N/A	Low
ATR-107	High	High	Intermediate	High	76%	High
Bococizumab	High	High	High	High	48%	High

CONCLUSION

- ISPRI analysis aligns better with the MAPPs data.
  - Using NetMHCIIpan-4.0, only 66 out of 112 (**59%**) peptides overlap with MAPPs.
  - Using EpiMatrix, 116 out of 126 (**92%**) peptides overlap with MAPPs.
- MAPPs alone generally overpredicts immunogenic risk.
- Neither NetMHCIIpan-4.0 nor MAPPs distinguishes regulatory versus effector epitope content, but ISPRI can.
- **ISPRI predictions provide consistent results as compared to studies combining three in vitro assays and public in silico tools.**
- **Fraction of the time and cost to obtain results with ISPRI.**

REFERENCES

Mattei, A. E., Gutierrez, A. H., Seshadri, S., Tivin, J., Ardito, M., Rosenberg, A. S., … De Groot, A. S. (2024). In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies. *mAbs*, *16*(1). https://doi.org/10.1080/19420862.2024.2333729

Wen-Ting K. Tsai, Yinyin Li, Zhaojun Yin, Peter Tran, Qui Phung, Zhenru Zhou, Kun Peng, Dan Qin, Sien Tam, Christoph Spiess, Jochen Brumm, Manda Wong, Zhengmao Ye, Patrick Wu, Sivan Cohen & Paul J. Carter (2024) Nonclinical immunogenicity risk assessment for knobs-into-holes bispecific IgG1 antibodies, mAbs, 16:1, 2362789, DOI: 10.1080/19420862.2024.2362789