MULTI-FACTORED IMMUNOGENICITY RISK ASSESSMENT APPROACH FOR BISPECIFIC IMMUNE-CELL ENGAGERS

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PURPOSE

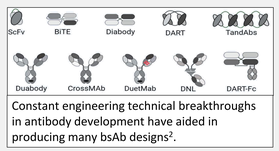
Detect and predict immunogenicity and Anti-Drug Antibodies (ADA) for bispecific antibodies

- Immunogenicity risk assessment has become an essential component of developability appraisal for biologic drug candidates including monoclonal (mAb), bispecific (bsAb), and multispecific (msAb) antibody constructs. The incidence of anti-drug antibody (ADA) formation is correlated with CD4+ T cell epitope content, which can be modeled using in silico tools.
- ISPRI, (Interactive Screening and Protein Re-engineering Interface) an in-silico toolkit developed by EpiVax, Inc., can rapidly assess the overall immunogenic potential of a biologic and identify T cell epitope clusters that may contribute to it.

OBJECTIVE

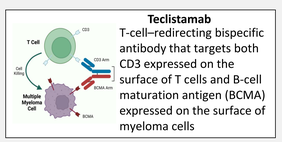
Describe the adaptation of an approach validated by mAb clinical data[1] to the analysis of immunogenic potential of msAbs, in which immuno-informatic tools are applied with consideration for clinical and mecha-nistic factors for a comprehensive estimation of immunogenic risk.

Focuses on immunogenicity risk assessment (IgRA) of bsAb-based immunotherapies for cancer, highlighting risk factors that need to be considered to understand the mechanistic root causes of immunogenicity.

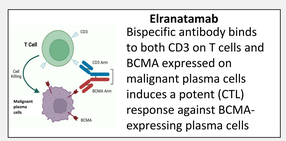


Constant engineering technical breakthroughs in antibody development have aided in producing many bsAb designs[2].

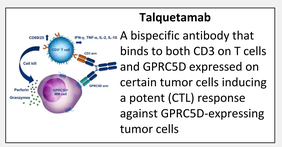
We retrieved amino acid sequences of six bsAb therapeutics for which immunogenicity data were available: **Teclistamab**, **Elranatamab**, **Talquetamab**, **Navicixizumab**, **Vanucizumab**, and **Amivantamab**.



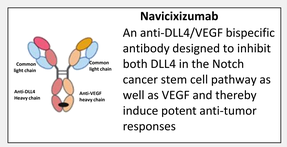
Teclistamab
T-cell–redirecting bispecific antibody that targets both CD3 expressed on the surface of T cells and B-cell maturation antigen (BCMA) expressed on the surface of myeloma cells



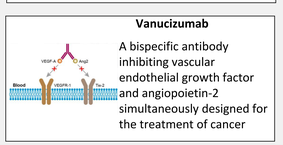
Elranatamab
Bispecific antibody binds to both CD3 on T cells and BCMA expressed on malignant plasma cells induces a potent (CTL) response against BCMA-expressing plasma cells



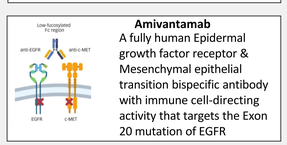
Talquetamab
A bispecific antibody that binds to both CD3 on T cells and GPRC5D expressed on certain tumor cells inducing a potent (CTL) response against GPRC5D-expressing tumor cells



Navicixizumab
An anti-DLL4/VEGF bispecific antibody designed to inhibit both DLL4 in the Notch cancer stem cell pathway as well as VEGF and thereby induce potent anti-tumor responses



Vanucizumab
A bispecific antibody inhibiting vascular endothelial growth factor and angiopoietin-2 simultaneously designed for the treatment of cancer

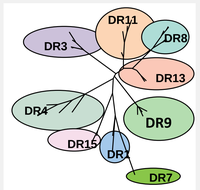


Amivantamab
A fully human Epidermal growth factor receptor & Mesenchymal epithelial transition bispecific antibody with immune cell-directing activity that targets the Exon 20 mutation of EGFR

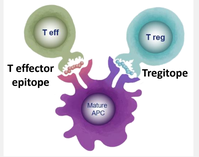
METHODS

- All sequences were analyzed using a representative of HLA-DR supertypes that cover >95% of the worldwide human population. **(A)**
- T cell epitopes were mapped for each complete protein sequence as well as their constituent domains using the EpiMatrix algorithm. **(B)**
- ISPRI distinguishes regulatory T cell epitopes (Tregitopes™) from T effector epitopes in the analysis of the immunogenic potential. JanusMatrix™ is able to compare T cell epitope clusters against human proteins to see if they are similar at the 2, 3, 5, 7, and 8 (TCR facing) positions of the nine-mer, which may cause recognition of these nine-mers as self by T cells. "Self-like" regions and Tregitopes™ may promote tolerance, giving a more accurate representation than volume of epitope content alone. **(C)**
- Immunogenicity scores are predicted and compared on a scale created from a large number of random sequences with amino acids at naturally occurring frequencies, normally distributed around zero in order to characterize the T-cell epitope content.

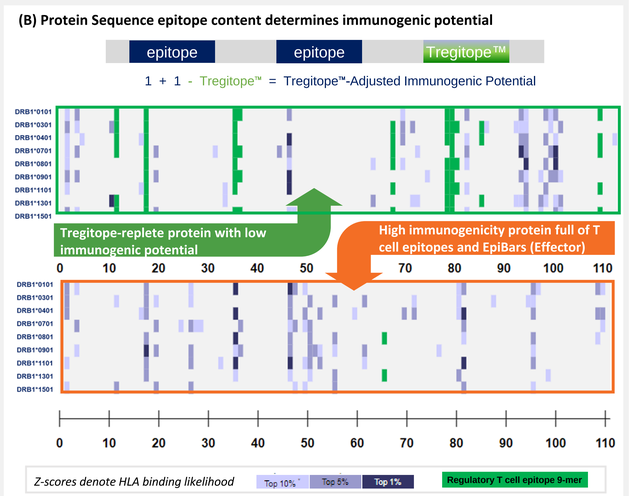
(A) HLA DR Supertypes offer broad global population coverage



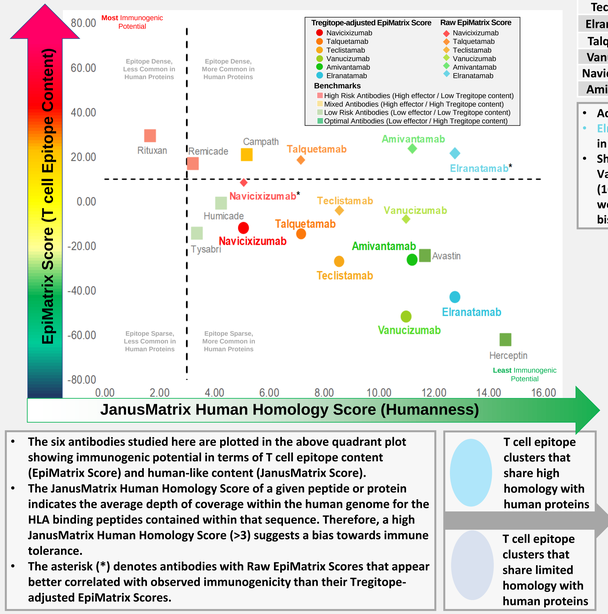
(C) T cell phenotype modulates immune response



(B) Protein Sequence epitope content determines immunogenic potential



RESULTS



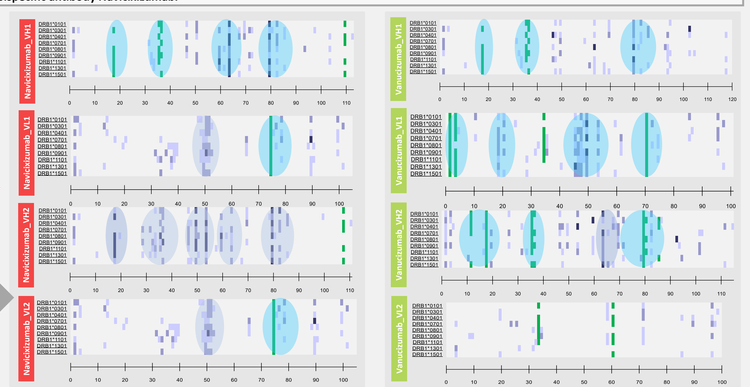
bsAb	Target 1	Target 2	Raw EpiMatrix		Tregitope-adjusted EpiMatrix		Observed ADA	Clinical Status
			Score	Predicted ADA	Score	Predicted ADA		
Teclistamab	BCMA on myeloma cells	CD3 on T cells	-3.81	9.27%	-27.05	1.32%	0.50%	FDA Approved
Elranatamab*	BCMA on plasma cells		21.63	27.40%	-42.86	0%	10.70%	Phase 2/3
Talquetamab	GPRC5D on tumor cells		18.59	24.71%	-14.39	4.64%	11%	Phase 2/3
Vanucizumab	Ang-2 (Angiopoietin)	VEGF -A	-7.8	7.30%	-51.76	0%	5%	Phase2 *(Discont'd)*
Navicixizumab*	Delta-like ligand 4	VEGF	8.71	16.90%	-11.87	3.45%	16%	Phase 2/3
Amivantamab	EGFR exon 20 mutation	MET	23.82	29.42%	-26.23	1.46%	1%	FDA Approved

- The six antibodies studied here are plotted in the above quadrant plot showing immunogenic potential in terms of T cell epitope content (EpiMatrix Score) and human-like content (JanusMatrix Score).
- The JanusMatrix Human Homology Score of a given peptide or protein indicates the average depth of coverage within the human genome for the HLA binding peptides contained within that sequence. Therefore, a high JanusMatrix Human Homology Score (>3) suggests a bias towards immune tolerance.
- The asterisk (*) denotes antibodies with Raw EpiMatrix Scores that appear better correlated with observed immunogenicity than their Tregitope-adjusted EpiMatrix Scores.

T cell epitope clusters that share high homology with human proteins

T cell epitope clusters that share limited homology with human proteins

- Additional variables are vital to understand the variation in observed ADA between molecules with similar targets & MOA
- Elranatamab's high observed ADA compared to the low ADA of Teclistamab, whose targets are the same, may be attributable to a difference in absolute epitope content (see Raw EpiMatrix Score) and/or abrogated Treg activity due to overwhelming Teff activation involving CD3.
- Shown below, Vanucizumab & Navicixizumab, both of which target VEGF, stimulated different degrees of ADA in the clinic. In this case, Vanucizumab (5% ADA) contains 11 T cell epitope clusters, 91% of which are well conserved in human sequences, whereas Navicixizumab (16% ADA) contains 13 T cell epitope clusters, 50% of which are not well conserved in human sequences. Furthermore, the blockade of DLL4 would be expected to diminish Treg induction & immune activity, possibly contributing to the observed high immunogenicity of the bispecific antibody Navicixizumab.



CONCLUSIONS

In this study, observed ADA incidence differs by ≤5% from ADA predicted by a traditional method for three of the six bispecific antibodies analyzed, indicating the need for consideration of additional factors to fully characterize immunogenic potential of innovative new multi-specific therapeutic candidates.

- Notably, clinical sample sizes were small, and differences were greatest, on average, for molecules with CD3-targeting domains, such as Elranatamab, potentially due to abrogated Treg activity and Teff escape from suppression through provision of potent costimulatory signals or other inflammatory cytokines [3]. In such cases, absolute epitope content (i.e., Raw EpiMatrix Score) may be an important indicator of immunogenic risk.
- The potential impact of Treg impairment is also visible in molecules that do not specifically target T cell surface markers, such as **Navicixizumab**, for which the blockade of DLL4 would be expected to diminish Treg induction and immune activity, possibly contributing to its observed high immunogenicity.
- EpiVax's ISPRI Toolkit not only allows for the rapid in silico analysis and assessment of the immunogenicity risk of complex, multidomain biologics, but also estimates ADA rates.
- This innovative, ground-breaking technology and tool for assessment will only increase in importance as biologics formats become more complex and it becomes increasingly necessary to consider the immunogenic potential of not only novel constructs as a whole (including at new junctions not found in nature), but also of their individual components with distinct T cell epitope characteristics.

REFERENCES

1. De Groot & Martin, Clin Immunol 2009, 131(2)
2. Fig. 1C. Wei et al., Front. Immunol., 28 October 2022 Volume 13 – 2022
3. Sojka DK, Huang YH, Fowell DJ. Immunology. 2008 May; 124(1)

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