ISPRI, Interactive Screening and Protein Reengineering Interface for Complex Therapeutic Modalities

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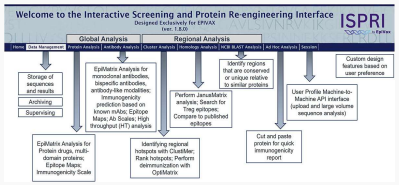
OVERVIEW

As biologics become more complex, immunogenicity remains a critical concern—even for therapies constructed from fully human sequences. Evaluating immunogenicity risk is essential in preclinical development and for regulatory review, as immune responses can reduce efficacy or cause adverse effects.

EpiVax has developed and applied an in silico platform, ISPRI (Interactive Screening and Protein Re-engineering Interface), to evaluate the immunogenic potential of biologics, such as bispecific T cell engagers (BiTEs). ISPRI rapidly identifies regions of immunogenic or tolerogenic potential based on T cell epitope content. Here, we applied ISPRI to Amgen's Pasotuxizumab molecule (AMG 212) to identify and visualize T cell epitope content, assess potential regulatory T cell involvement, and demonstrate ISPRI's utility in early immunogenicity risk assessment for next-generation therapies.



APPROACH

Step by step process flow

1. **EpiMatrix** for determining HLA binding potential **GLOBAL analysis**
   - *A prerequisite for T cell activation*
2. **Tregitope Identification** accounts for validated regulatory epitopes
   - *Evaluation of regulatory potential*
3. **ClustiMer** for identifying T cell epitope clusters **REGIONAL analysis**
   - *Potential targets for deimmunization*
4. **JanusMatrix** for assessing cross-conservation with self
   - *Downgrade immunogenic potential of clusters based on human homology*
5. **Basic Homology** analysis
   - *Cross-reference each cluster against GenBank and IEDB*
6. **Clinical Contextual Analysis**
   - *Consider other risk factors including process development related quality attributes and patient associated factors.*

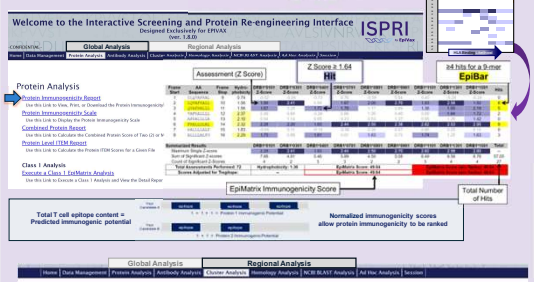
REFERENCES

- Mattei, Aimee E et al. "In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies." mAbs vol. 16,1 (2024): 2333729. doi:10.1080/19420862.2024.2333729
- Penny, Hweixian Leong et al. "Characterization and root cause analysis of immunogenicity to pasotuxizumab (AMG 212), a prostate-specific membrane antigen-targeting bispecific T-cell engager therapy." Frontiers in immunology vol. 14 1261070. 23 Oct. 2023, doi:10.3389/fimmu.2023.1261070

TOOL DESCRIPTION

Identify & Quantify HLA Ligands
EpiMatrix™

EpiMatrix™
A matrix-based algorithm that predicts linear T cell epitopes by assessing their binding potential to nine HLA-DR supertype alleles, a critical prerequisite for immunogenicity

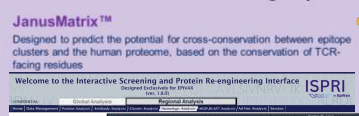


ClustiMer™
Identifies T cell epitope clusters—short sequences likely to bind multiple HLA alleles—that can trigger strong immune responses even in proteins with low overall epitope content

T cell epitopes are not randomly distributed, but instead tend to cluster

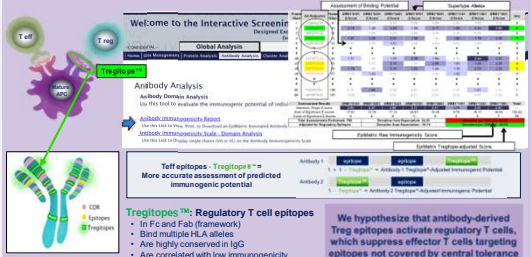
Adjust for the Humanness of epitopes
JanusMatrix™ & Tregitope™-Adjustment

JanusMatrix™
Designed to predict the potential for cross-conservation between epitope clusters and the human proteome, based on the conservation of TCR-facing residues



Depth of cross-conservation is an indicator of tolerated or regulatory potential

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



Tregitopes™: Regulatory T cell epitopes
- In Fc and Fab (framework)
- Bind multiple HLA alleles
- Are highly conserved in IgG
- Are correlated with low immunogenicity
- May be present in other self proteins

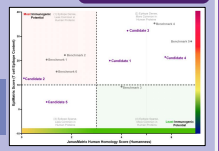
We hypothesize that antibody-derived Treg epitopes activate regulatory T cells, which suppress effector T cells targeting epitopes not covered by central tolerance

Evaluation of Immunogenic Potential
Bringing the concepts together

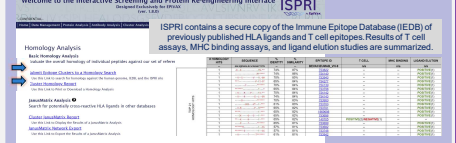
Applying Human Intelligence More than a Score

ISPRI platform's Quadrant Plot
A visual framework for comparing the immunogenic potential of therapeutic candidates.

This two-dimensional visualization helps categorize candidates by risk level, enabling rapid, comparative assessment across a panel of molecules or constructs.



Alignment to published epitopes IEDB

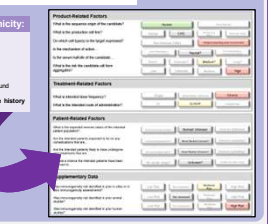


ISPRI contains a secure copy of the Immune Epitope Database (IEDB) of previously published HLA ligands and T cell epitopes. Results of T cell assays, MHC binding assays, and ligand elution studies are summarized.

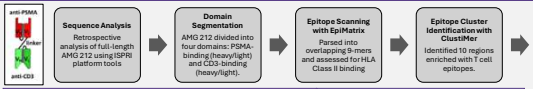
Factors contributing to immunogenicity:

Product Attributes	Treatment
AA Sequence	Dose
Target	Route
Mechanism of Action	Frequency
Foreignness	Duration
Structure	**Patient**
Post-translational modifications	Genetic Background
Aggregation	HLA
Impurities	Disease/Immune history
Formulation	Co-medication

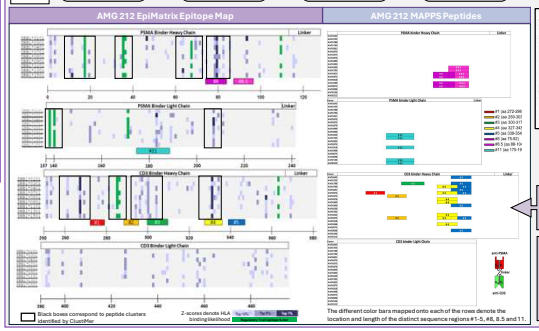
Clinical context questionnaire to aid the interpretation of the ISPRI-generated data through the lens of the indication, mechanism of action, and other important considerations.



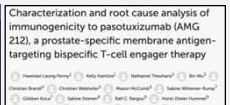
APPLICATION CASE STUDY: BiTE



MAPPs and ISPRI both identified several non-tolerogenic epitopes that could have contributed to the immunogenicity in the SC dosed patients.



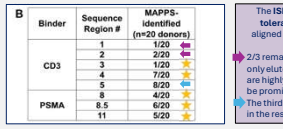
Characterization and root cause analysis of immunogenicity to pasotuxizumab (AMG 212), a prostate-specific membrane antigen-targeting bispecific T-cell engager therapy



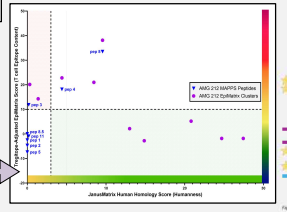
Eight regions were identified through MAPPS that correspond to peptides naturally processed and presented by antigen-presenting cells from the AMG 212 molecule.

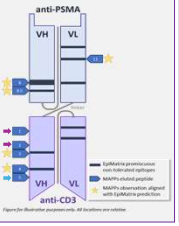
EpiMatrix Epitope Maps with ClustiMer defined clusters (left) are compared to the MAPPs eluted peptide data reported by Penny et al. (right).

ClustiMer-defined and reported MAPPs eluted peptides were scored with EpiMatrix and JanusMatrix and plotted on the Immunogenicity Quadrant Plot to determine the relative immunogenic risk



Binder	Sequence Region #	MAPPS-Identified (n=20 donors)
CD3	1	1/20
	2	2/20
	3	1/20
	4	7/20
	5	8/20
PSMA	8	4/20
	8.5	6/20
	11	5/20





The **ISPRI toolkit predicted several non-tolerant T cell epitopes** that were well-aligned (5/8) to the published MAPPs eluted peptides.
2/3 remaining eluted peptides (#1&2) were only eluted by 1-2 donors, indicating that they are highly HLA-restricted and not expected to be promiscuous T cell epitopes.
The third (#5) failed to confer T cell reactivity in the restimulated T cell line assay.

CONCLUSION

- Carefully validated in silico tools—such as those available on the ISPRI platform—can predict both immunogenicity (via EpiMatrix) and tolerogenic potential (via JanusMatrix).
- ISPRI tools are well-suited for evaluating the immunogenic risk of complex antibody formats by analyzing individual arms or components that may harbor unique T cell epitopes.
- ISPRI predictions show strong alignment with MAPPs-eluted peptides and other in vitro methods, while offering faster turnaround and early-stage actionable insights.
- Leveraging both in silico and in vitro orthogonal approaches enables researchers to improve efficiency, reduce preclinical development costs, and make better-informed immunogenicity risk decisions.