Prospectively Assessing the Immunogenic Risk of Potential Synthetic Peptide Impurities *in silico* with the What-if Machine (WhIM)

Aimee Mattei MS[1], Brian J Roberts PhD[1], Matthew Ardito[1], William Martin[1], and Anne S De Groot MD[1,2]

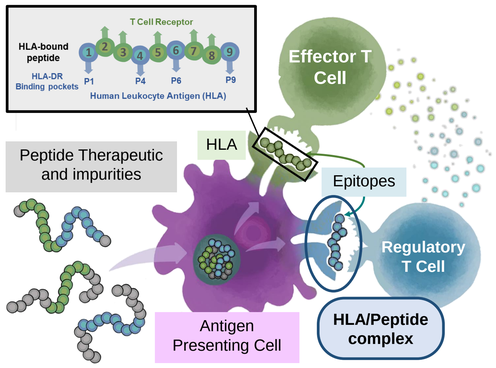
[1]EpiVax, Inc.; [2]CEO/CSO EpiVax, Inc., Professor (Research), University of Georgia

What is WhIM?

The **What-if Machine (WhIM)** is an algorithm that, for a given input peptide sequence, **models** (in silico) nearly all **impurities** that may occur during peptide manufacturing and storage, such as amino acid deletions, insertions, racemization, and side chain modifications.[1]

WhIM **generates a comprehensive list** of thousands of theoretical impurities, depending on sequence length.

The generated impurity sequences are scored with well-established immunoinformatics tools, **EpiMatrix** for immunogenic potential and **JanusMatrix** for humanness, at both an **overall** and an **impurity-specific** level.



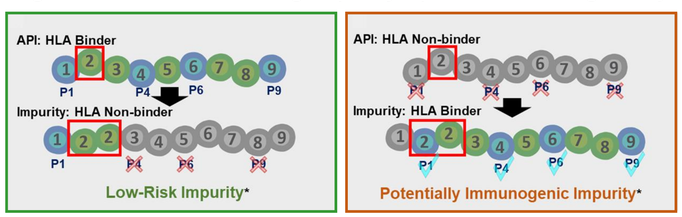
EpiMatrix
"what the HLA will present"
identifies putative T cell epitopes

JanusMatrix
"what the T cell will see"
Human (tolerated) vs.
Foreign (immunogenic)

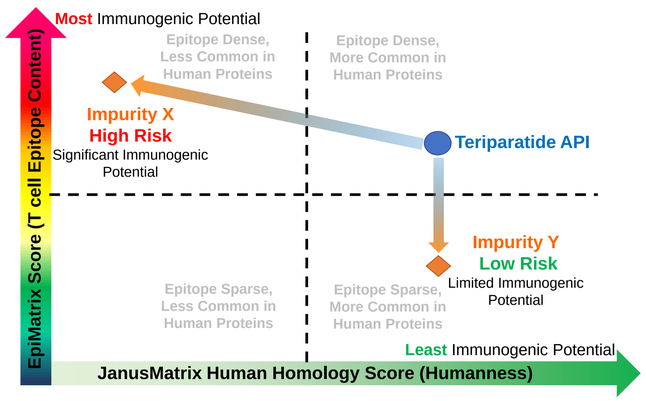
Impurities Can Introduce Immunogenic Risk

Impurities can **eliminate existing** or **create new** **T cell epitope content** relative to the API peptide sequence.

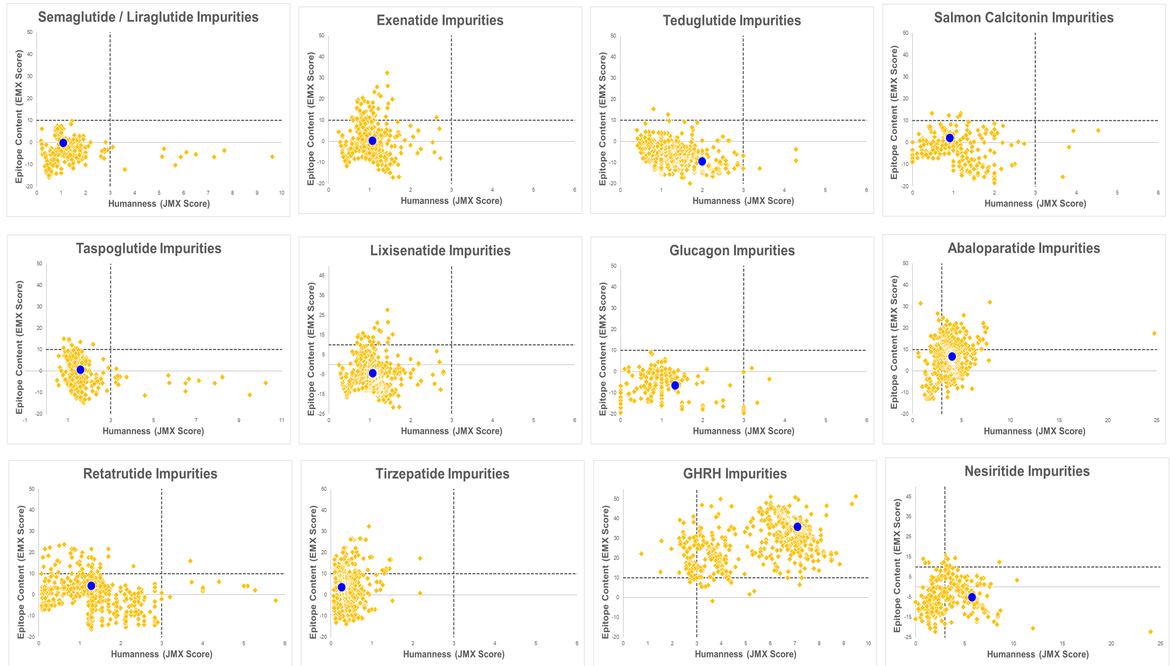
Impurities that create *foreign* new epitope content carry the highest risk for introducing impurity-specific immunogenicity.[2]



*Risk above based on HLA binding potential only



Impurity Immunogenicity Risk Profiles for Peptide Therapeutics



WhIM-generated impurity immunogenicity risk profiles are shown above for 12 peptide APIs. **API baseline peptides are shown in blue** and **WhIM-generated theoretical impurities are shown in gold**. EpiMatrix Scores >+10 indicate significant epitope content and significant potential for immunogenicity. JanusMatrix Scores >3 indicate significant cross-conservation with epitopes derived from the human proteome and therefore significant tolerance potential. Impurities that either increase epitope content (move up) or reduce humanness (move left) relative to their API have the highest risk of introducing impurity-driven immunogenicity.

Conclusions

- For a given peptide, WhIM provides a comprehensive interactive list of theoretical impurities and a summarized risk profile for the generation of immunogenic impurities.
- WhIM can be used *proactively* to ensure proper manufacturing procedures are in place to limit the generation of high-risk impurities, saving resources in the development of safe and effective novel or generic peptide therapeutics.
- Regulators or sponsors can *retrospectively* investigate the immunogenic potential of specific impurities identified in the drug product using WhIM.
- It is recommended that WhIM be used in conjunction with in vitro HLA binding and T cell assays, which serve to validate the predicted immunogenic sequences if they are in fact identified in the drug product.

Selected WhIM-identified High Risk Teriparatide Impurities were Immunogenic In Vitro

Teriparatide API

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

Promiscuous T cell epitope that has shared TCR-face with epitopes in the human proteome

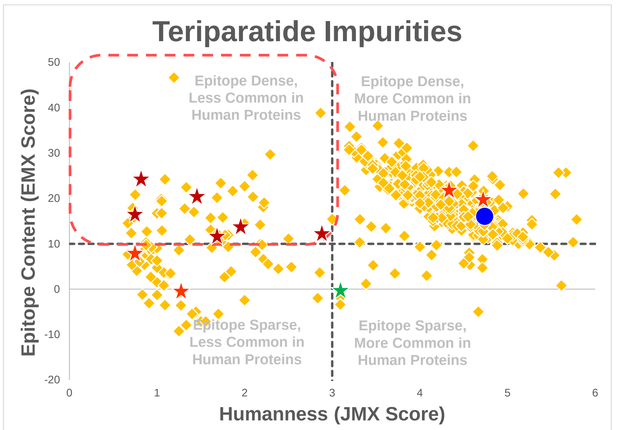
EpiMatrix	16.03	**High** Score indicates significant epitope content
JanusMatrix	4.74	**High** Score indicates high potential for tolerance

WhIM Impurities

Most **impurities** have significant epitope content (EMX>10) and decreased human cross-conservation relative to the **API.**

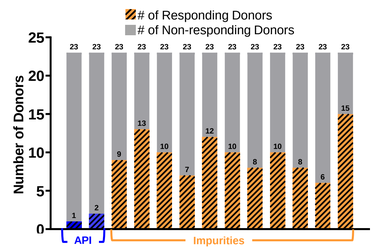
Synthetic teriparatide carries a **high risk** for generating immunogenic impurities, especially modifications within the tolerogenic promiscuous epitope region.

Highest risk zone (EMX >10, JMX < 3)



Confirmatory T Cell Assays

The **teriparatide API** peptide was *tolerated* in most donors, as predicted in silico.



Selected teriparatide **impurities** (★) impacting the tolerogenic promiscuous epitope region and/or introducing predicted new epitope content were more immunogenic than the API at equal concentrations, as predicted in silico.

References

1. D'Hondt M, Bracke N, Taevernier L, Gevaert B, Verbeke F, Wynendaele E, De Spiegeleer B. Related impurities in peptide medicines. *J Pharm Biomed Anal.* 2014 Dec;101:2-30. doi: 10.1016/j.jpba.2014.06.012. Epub 2014 Jun 13. PMID: 25044089
2. De Groot AS, Roberts BJ, Mattei A, Lelias S, Boyle C, Martin WD. Immunogenicity risk assessment of synthetic peptide drugs and their impurities. Drug Discov Today. 2023 Jul 17;28(10):103714. doi: 10.1016/j.drudis.2023.103714. Epub ahead of print. PMID: 37467878.

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