# Rapid and Predictive Immunogenicity Risk Assessments Using In Silico Methods

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## PURPOSE

The incidence of anti-drug antibody (ADA) formation is correlated with CD4+ T cell epitope content, which can be modeled using in silico tools. Our group has developed comprehensive in silico methods for identifying T effector and regulatory T cell epitopes (Tregitopes) in monoclonal antibodies, enabling a rapid and accurate forecast of immunogenic potential.

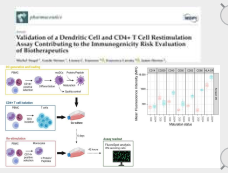
## OBJECTIVE

In this study, our immunoinformatic tools were retroactively applied to a set of 24 biotherapeutics originally presented by Siegel M, et al. We compared reported rates of ADA formation to rates predicted by our in silico algorithms and to rates observed in DC:CD4+ T cell restimulation assays.

## METHODS

**In Vitro DC:CD4+ T cell restimulation assay**

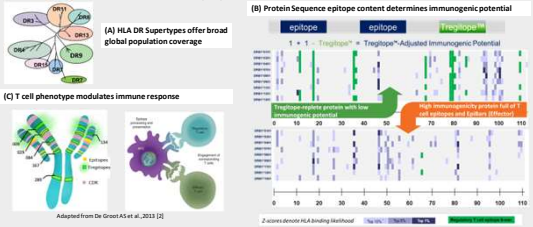
The study conducted by Siegel et al. (2022) applied the DC:CD4+ T cell restimulation assay to 24 biotherapeutics, resulting in T cell responses predictive of clinical ADA rates [1].



- Isolate monocytes from healthy donor PBMCs.
- Load protein onto monocyte-derived dendritic cells (moDC).
- Mature moDC then isolate autologous CD4+ T cells.
- Co-culture CD4+T cells with loaded moDCs.
- Add isolated monocytes challenged with same protein.
- Measure IFN-γ production by FluoroSpot after incubation.
- Assessment of T-cell response.

**In Silico Assessment of T cell epitope Content and Tregitope Content with ISPRI**

**(A)** Sequences were analyzed using a representative of a set of HLA-DR supertypes that cover >95% of the worldwide human population.
**(B)** T cell epitopes were mapped for each variable domain sequence using the EpiMatrix algorithm.
**(C)** ISPRI distinguishes regulatory T cell epitopes (Tregitopes™) from T effector epitopes in the analysis of the immunogenic potential. Tregitopes™ are highly conserved T cell epitopes derived from IgG that activate regulatory T cells and promote tolerance induction to associated antigens. Adjusting the score for their presence gives a more accurate representation than the volume of epitope content alone.

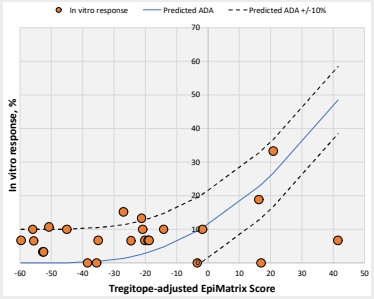



**In Silico Modeling of Anti-Therapeutic Immune Response with ISPRI**

- EpiVax has developed a regression model to relate Tregitope-adjusted EpiMatrix Scores to observed rates of anti-therapeutic antibody formation.
- This model was developed using 22 licensed monoclonal antibody products.
- This model can be used to prospectively predict ADA rates from the VH+VL Tregitope-adjusted EpiMatrix Score of a given biotherapeutic.

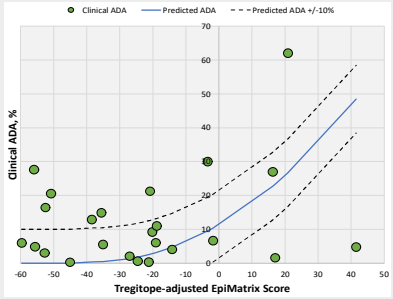
## RESULTS

**Tregitope-adjusted EpiMatrix Score vs. In vitro response**




Correct predictions within 10% range = 79.2% (19/24)

**Tregitope-adjusted EpiMatrix Score vs. Clinical ADA**



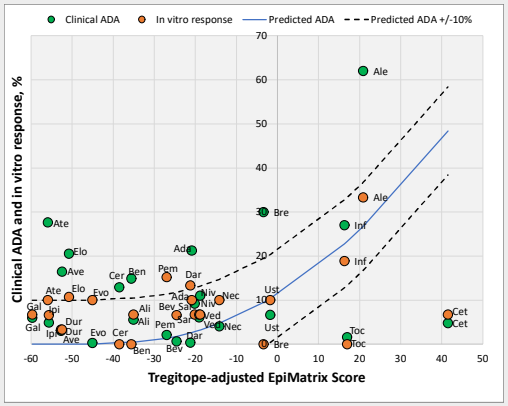

Correct predictions within 10% range = 58.3% (14/24)

The in silico ADA prediction model (blue line) aligns within a 10% window (black dashed lines) to in vitro observations (orange circles) for 19/24 biotherapeutics and to reported clinical ADA (green circles) for 14/24 cases.

| Antibody Name | Target | Clinical ADA% | In vitro Response (% of positive donors; n=30) | EpiVax Predicted ADA% |
|---|---|---|---|---|
| Alemtuzumab (Ale) | CD52 | 62.0 | 33.3 | 26.7 |
| Brentuximab (Bre) | CD30 | 30.0 | 0.0 | 9.5 |
| Atezolizumab (Ate) | PD-L1 | 27.6 | 10.0 | 0.0 |
| Infliximab (Inf) | TNF-α | 27.0 | 18.9 | 22.8 |
| Adalimumab (Ada) | TNF-α | 21.3 | 10.0 | 2.6 |
| Elotuzumab (Elo) | SLAMF7 | 20.5 | 10.7 | 0.0 |
| Avelumab (Ave) | PD-L1 | 16.5 | 3.3 | 0.0 |
| Benralizumab (Ben) | CD125 | 14.9 | 0.0 | 0.5 |
| Certolizumab (Cer) | TNF-α | 12.9 | 0.0 | 0.4 |
| Nivolumab (Niv) | PD-1 | 11.0 | 6.7 | 3.2 |
| Sarilumab (Sar) | IL-6R | 9.2 | 6.7 | 2.8 |
| Ustekinumab (Ust) | IL-12/IL-23 | 6.6 | 10.0 | 10.4 |
| Vedolizumab (Ved) | Integrin α4β7 | 6.0 | 6.7 | 3.1 |
| Galcanezumab (Gal) | Calcitonin | 6.0 | 6.7 | 0.0 |
| Alirocumab (Ali) | PCSK9 | 5.5 | 6.7 | 0.5 |
| Ipilimumab (Ipi) | CTLA-4 | 4.9 | 6.6 | 0.0 |
| Cetuximab (Cet) | EGFR | 4.8 | 6.7 | 49.4 |
| Necitumumab (Nec) | EGFR | 4.1 | 10.0 | 4.7 |
| Durvalumab (Dur) | PD-L1 | 3.0 | 3.3 | 0.0 |
| Pembrolizumab (Pem) | PD-1 | 2.1 | 15.2 | 1.3 |
| Tocilizumab (Toc) | IL-6R | 1.6 | 0.0 | 29.4 |
| Bevacizumab (Bev) | VEGF | 0.6 | 6.6 | 1.8 |
| Daratumumab (Dar) | CD38 | 0.4 | 13.3 | 2.6 |
| Evolocumab (Evo) | PCSK9 | 0.3 | 10.0 | 0.0 |

- The in silico ADA prediction model aligns with in vitro observations but not with the reported clinical ADA for Ate, Ada, Ale, Ave, Bre, Ben, and Cer.
- The in silico ISPRI ADA prediction model successfully predicted clinical ADA for Pem and Dar but did not align with in vitro observations for these two antibodies.
- In vitro and clinical models demonstrate alignment for three (Cet, Toc, and Elo) out of the 24 biologics (12.5%), but these fall outside the 10% boundaries for the In silco model.

**Tregitope-adjusted EpiMatrix Score vs. Clinical ADA and In vitro response**




Correct predictions within 10% range = 87.5% (21/24)

## CONCLUSIONS

- As this case study illustrates, in silico ADA predictions using the ISPRI tool were consistent (+/- 10%) with the reported DC:CD4+ T cell restimulation in vitro observations for 19 out of the 24 biotherapeutics (79%). In silico ISPRI ADA predictions were highly correlated with in vitro and/or clinical ADA rates with 21 out of 24 correctly predicted within a 10% window (87.5%).
- In silico methodologies empower researchers with the capability to rapidly and efficiently process thousands of sequences in mere minutes. This computational approach offers a level of predictability comparable to in vitro assessments, all while significantly reducing time and cost expenditures.
- In contrast, in vitro experimentation is often challenging to conduct in a high-throughput manner, primarily due to its time-consuming nature and substantial associated costs.
- Recognizing the importance of a multi-faceted approach, it's imperative to identify when in vitro and in silico methods can be most effectively utilized to enhance time, cost efficiency, and strategic development in research. Further studies of this type support deimmunization, humanization, and other approaches to de-risking antibody therapeutics in our interactive in silico screening and optimization platform (ISPRI).

## REFERENCES

[1] Siegel M, Steiner G, Validation of a Dendritic Cell and CD4+ T Cell Restimulation Assay Contributing to the Immunogenicity Risk Evaluation of Biotherapeutics. Pharmaceutics. 2022 Dec 1;14(12):2672. doi: 10.3390/pharmaceutics14122672. PMID: 36559166; PMCID: PMC9781343.

[2] De Groot AS, Terry F, Cousens L, Martin W. Beyond Humanization and De-immunization: Tolerization as a Method for Reducing the Immunogenicity of Biologics. Exp Rev Clin Pharm. 2013;6(6):651-52.

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