Immune Tolerance-Adjusted Personalized Immunogenicity Prediction for Rare Diseases

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Abstract

Background. Gene-deficiency diseases are treatable either by replacement of the missing protein or by gene therapy. Both types of interventions carry a risk of unwanted immune response to the therapeutic intervention (immunogenicity) due to the underlying deficiency. T cell responses to T cell epitope sequences in the therapeutic protein sequence that differ from the individuals' native protein are most likely to drive immune response. HLA also plays a role.

Approach. We have developed tools (EpiMatrix, ClustiMer, iTEM and JanusMatrix) to evaluate and assess the risk of immune response to protein or gene-replacement therapy using genotype and HLA DR type as input variables. We applied this system **P**ersonalized **Im**munogenicity Risk **A**ssessment **(PIMA)** to data for a cohort of Infantile-onset Pompe disease (IOPD) patients. IOPD is a glycogen storage disease caused by a deficiency of acid alpha-glucosidase (GAA). PIMA assesses the risk of immunogenicity based on the individual's unique GAA mutations and their HLA DR haplotype, while also correcting for "self-like" tolerized epitopes in the PIMA calculations. For this case study we focused on IOPD.

Results. Using the tolerance-adjusted version of PIMA in a logistic regression model with data from 48 cross-reactive immunological material (CRIM)-positive IOPD subjects, those with PIMA scores greater than 10 were 4-fold more likely to develop anti-drug antibodies ($p<0.03$) than those that had scores less than 10. Furthermore, we identified some GAA T cell epitopes that may be immunomodulatory. We hypothesize that Pompe patients having intact regulatory epitopes within their endogenous GAA would have an advantage since they would have GAA-specific T regulatory cells thereby conferring tolerance to the therapeutic GAA. Understanding the potential regulatory content of a therapeutic gene is highly relevant to enzyme deficiency diseases especially when considering treatment in the context of the patient's unique genotype.

Future Developments. The PIMA tool will integrate a Treg-adjusted score to improve individualized risk assessment that could improve decision-making regarding initiation of immune modulation for some IOPD patients. While the PIMA development presented here focused on IOPD, this platform utilizing a personalized approach will have a potential utility for other rare lysosomal and enzyme deficiency diseases where gene replacement is necessary.

Background

Impact of ADA in clinical outcome

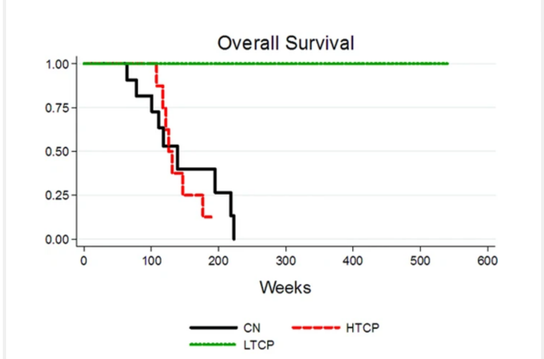


Figure 1. Antibody development in IOPD impacts clinical outcome. Kaplan-Meier curves for overall survival for CRIM-negative **(CN - black)** (n=11), High-titer CRIM-positive **(HTCP-red)** (n=9), and Low-titer CRIM-positive **(LTCP - green)** (n=14). *Figure from Banugaria et al., [1]*

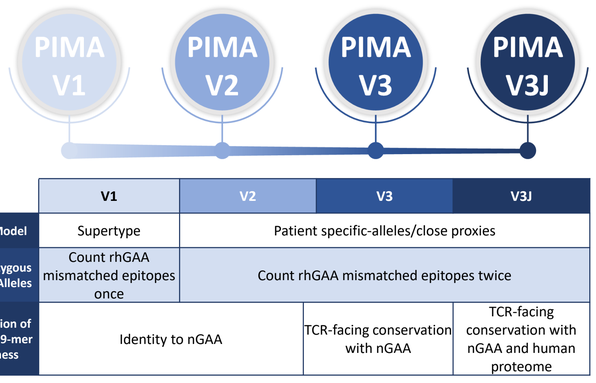
IOPD, a rare glycogen storage disease, necessitates enzyme replacement therapy (ERT) with recombinant GAA (rhGAA) for survival. Many patients develop anti-drug antibodies (ADA), rendering the therapy ineffective.

- **Cross-reactive immunologic material (CRIM)-negative patients**, with complete absence of endogenous GAA, mount high antibody titers that are associated with poor clinical outcomes when treated with ERT alone. The current standard of care for CRIM-negative IOPD patients is Immune Tolerance Induction (ITI), which involves ab immunosuppressive regime.
- **CRIM-positive patients** are expected to be immune tolerant to ERT, however, **over one-third** can develop treatment-limiting ADA. There is currently no effective method for determining which CRIM-positive patients are likely to develop treatment-limiting ADA, and therefore justify the risk of immunosuppressive therapies.

Methods

PIMA Development

- PIMA, a personalized immunoinformatic analysis developed to predict ADA risk in CRIM-positive IOPD patients.[2]
- Novel versions of the PIMA Tool (V2, V3, and V3J) were developed using the computational tool EpiMatrix to identify CD4-stimulating T-cell epitopes in rhGAA.[3]
- Version V3J incorporates JanusMatrix[4] to identify both effector and potentially tolerated or regulatory GAA T cell epitopes.
- Putative regulatory epitopes identified using JanusMatrix were tested in Tetanus-Toxoid Bystander Suppression assays (TTBSA).

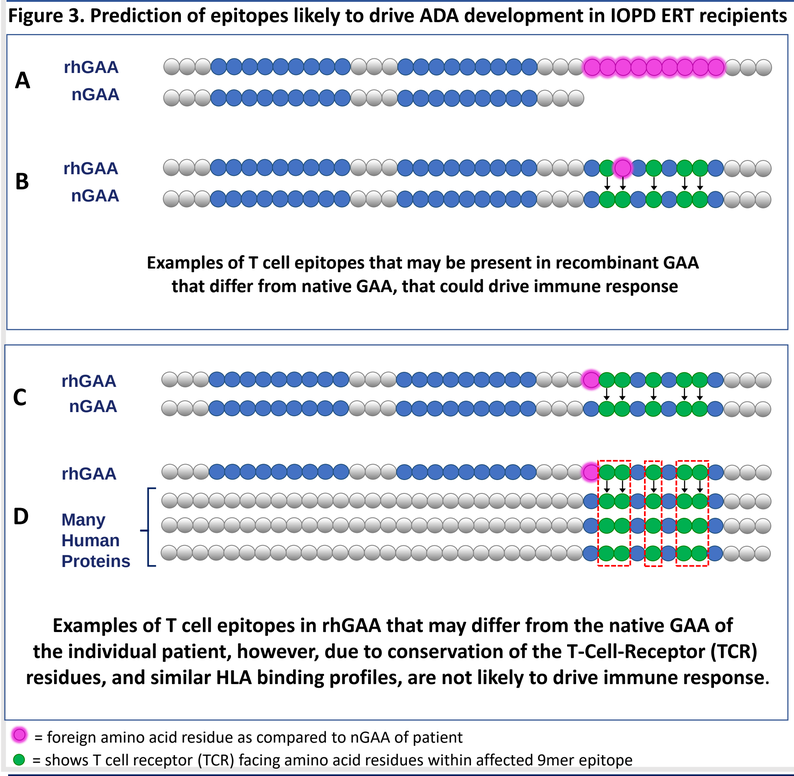


	V1	V2	V3	V3J
HLA Model	Supertype	Patient specific-alleles/close proxies		
Homozygous nGAA Alleles	Count rhGAA mismatched epitopes once	Count rhGAA mismatched epitopes twice		
Definition of rhGAA 9-mer selfness	Identity to nGAA		TCR-facing conservation with nGAA	TCR-facing conservation with nGAA and human proteome

Figure 2. The evolution of the PIMA algorithm. New-generation PIMA methods have been refined to improve the overall predictive accuracy. Versions vary based on several criteria 1) the type of HLA model used, 2) how homozygous alleles are handled in scoring, and 3) the definition of selfness used to identify tolerated epitopes.

Epitope differences in therapeutic GAA from endogenously expressed native GAA predicted to drive ADA or be tolerized

Figure 3. Prediction of epitopes likely to drive ADA development in IOPD ERT recipients



Examples of T cell epitopes that may be present in recombinant GAA that differ from native GAA, that could drive immune response

Examples of T cell epitopes in rhGAA that may differ from the native GAA of the individual patient, however, due to conservation of the T-Cell-Receptor (TCR) residues, and similar HLA binding profiles, are not likely to drive immune response.

= foreign amino acid residue as compared to nGAA of patient

= shows T cell receptor (TCR) facing amino acid residues within affected 9mer epitope

Epitopes that drive immune response

T cell epitopes within the rhGAA may be recognized as "foreign" if they are:

A. Located within the truncated or mutated portions of the patient-specific nGAA.

B. Located within the rhGAA that contain cell receptor (TCR)-facing residues that are different from those found in the nGAA.

Epitopes that are tolerated

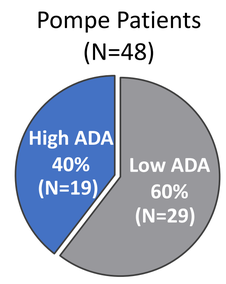
C. T cell epitopes within the rhGAA that contains different MHC-facing residues but the same TCR-facing residues as epitopes found in nGAA are predicted to be tolerated by the immune system (this hypothesis was included in PIMA V3).

D. The presence of a T cell epitope in the rhGAA sequence with TCR-facing residues highly cross-conserved with several self-human proteins may also be tolerated by the immune system. (This hypothesis was included in PIMA V3J).

Results

PIMA Score as Predictors of ADA Status

- rhGAA and nGAA sequences of 48 CRIM positive IOPD patients with known GAA variants, HLA type, and ADA titers were analyzed using the four PIMA algorithms.
  - ❑ *The PIMA scores reflect predicted T-cell epitopes within the rhGAA sequence that are foreign to the patient, given their nGAA sequence across both alleles.* At a PIMA Score of +10 (established threshold for developing ADA) accurately-predicted ADA status ranged from 54% to 64%, the highest percent predicted by PIMA V3J. [**Figure 4**].
- At a PIMA Score of +10 (established threshold for developing ADA) accurately-predicted ADA status ranged from 54% to 64%, the highest percent predicted by PIMA V3J. [**Figure 4**].
- PIMA V3J registers the highest odds ratio as a predictor of high ADA development in IOPD in a univariate and multivariable logistic regression analysis.
  - ❑ Patients with PIMA scores >10 were 4.12 times more likely to develop high ADA ($p<0.0246$) [**Table 1**].
  - ❑ Odds of developing high ADA improved with age at initiation of ERT as a covariate, with the highest adjusted OR of 4.40 ($p<0.0296$) [**Table 2**].



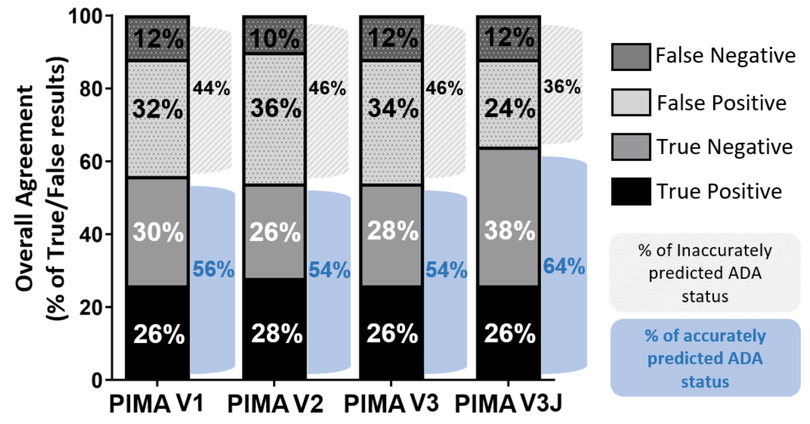


Figure 4. Overall agreement of the four PIMA scoring algorithms as predictors of ADA status.

Table 1. Univariate logistic regression

PIMA	iTEM OR (95% CI)	P-value
PIMA V1	2.32 (0.71-8.21)	0.1728
PIMA V2	2.27 (0.67-8.58)	0.1997
PIMA V3	2.02 (0.62-7.14)	0.2544
PIMA V3J	4.12 (1.24-15.01)	0.0246*

Table 2. Multivariable logistic regression (Age in weeks at ERT as a covariable)

PIMA	PIMA OR (95% CI)	P-value	Age OR (95% CI)	P-value
PIMA V1	3.45 (0.93-15.04)	0.0770	1.07 (1.02-1.13)	0.0105*
PIMA V2	3.40 (0.87-16.23)	0.0945	1.07 (1.02-1.13)	0.0110*
PIMA V3	2.74 (0.75-11.54)	0.1418	1.07 (1.02-1.13)	0.0123*
PIMA V3J	4.40 (1.21-18.21)	0.0296*	1.06 (1.01-1.22)	0.0214*

Including age at initiation of ERT as a covariate improves the accuracy of logistic regression models

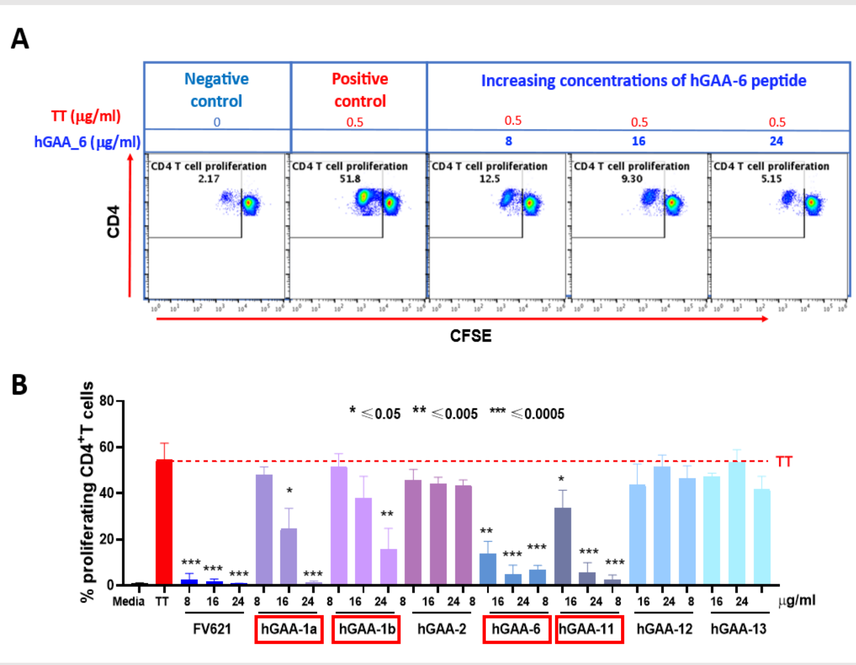
Putative Regulatory GAA-derived Peptides

- Twenty-one GAA-derived peptides were selected based on the cross-conservation of their T cell epitope content with human proteins.
- All peptides were validated using in vitro HLA-binding assays, and subsequently tested for their potential to inhibit CD4+ T cell proliferation and activation in healthy donor PBMCs.
  - ❑ *Tetanus Toxoid Bystander Suppression Assay (TTBSA) measures the inhibitory capacity of peptides on the recall response of human CD4 T cells to the TT antigen* [5]
- Significant suppressive capacity of CD4+ T cell proliferation was observed for **4 putative Treg peptides in GAA confirming their regulatory potential** across all donors tested [**Figure 5**].

Figure 5. GAA-derived peptide inhibition on CD4 T cell proliferation in TTBSA .

A. Representative flow cytometry dot plots show CD4 memory T cell proliferative response to tetanus toxoid (TT) and dose-dependent inhibition by hGAA-6 peptide.

B. PBMCs from healthy donors were stimulated with 0.5 µg/ml of TT with or without FV621 (Tregitope positive control) or GAA-peptides and analyzed at six days post-stimulation by flow cytometry for inhibition of CD4+ T cell proliferation. Data are the representative donor from 5 donors in the experiments. Four GAA-peptides demonstrate significant suppression of T cell proliferation. P values represent statistical significance between peptide stimulation vs TT using a two-tailed t test.



Conclusions & Future Directions

- This work supports preliminary evidence of the relationship between patient HLA, patient-specific GAA variants, and likelihood for high ADA response.
- The discovery of putative regulatory peptides in GAA is a key finding as these epitopes may be involved in natural tolerance to protein replacement therapies. Future integration of a Treg-adjusted score could improve individualized risk assessment.
- Given the potential for ADA in other lysosomal storage disorders and other conditions that require protein replacement and gene therapy, this model has the potential to identify high-risk patients and guide the optimal implementation of ITI for mitigating these challenges.
- The PIMA risk-assessment tool will provide clinicians with an individualized assessment of a patient's risk for developing treatment-limiting ADA based on their HLA haplotype and nGAA mutations, improving decision-making regarding ITI treatment.

EpiVax

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