When MFI misleads: allele-level discordance reveals apparent self-reactivity in a novel HLA-A*74 variant

Matthew Cusick[1], Kyle Jennings[1], Danielle Ross[1], Maria Ramirez[1], Matthew Najor[1], Jacob Collard[2], Rico Buchli[2]

1. Pathology, University of Michigan, Ann Arbor, MI, United States. 2. HLA Protein Technologies Inc., Oklahoma City, OK, United States

BACKGROUND

Why is this patient reacting against their own HLA-A*74?

- Sensitized transplant candidate with apparent anti-A*74 SAB reactivity
- Patient initially typed as HLA-A*74
- Moderate-to-strong A*74 bead signal created apparent self-reactivity
- Central possibilities:
  - True autoantibody
  - Denatured-antigen/bead artifact
  - Allele-level discordance
  - Cross-reactive alloantibody

Why MFI/ Luminex could not answer the question

- MFI is semi-quantitative
- Bead antigen may not represent the patient's allele
- Shared epitopes can create cross-reactivity
- Signal intensity does not identify the immunizing antigen
- MFI does not distinguish binding potency from maximal binding capacity

DESIGN

Apparent anti-self A*74 reactivity	SAB pattern and dilution
High-resolution NGS	GenDx/ Illumina- short read
Long-read confirmation	GenDx/ Oxford Nanopore- long read
Quantitative binding profile	**EC50 and OD90 profiling – HLA Protein Technologies Inc.**
Inhibition and reduction profiling	**Soluble-HLA inhibition and reductive profiling- HLA Protein Technologies Inc.**
Identity antibody origin	Epitope/eplet comparison

RESULTS

Figure 1. The patient does not express a canonical A*74:01 cell-surface molecule

A7401 341AA

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWD
QETRNVKAHSQTDRVDLGTLRGYYNQSEAGSHTIQMMYGCDVGPDGRLLRGYQQDAYDGKD
YIALNEDLRSWTAADMAAQITQRKWEAARVAEQLRAYLEGTCVEWLRRYLENGKETLQRTD
APKTHMTHHAVSDHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKW
ASVVVPSGQEQRYTCHVQHEGLPKPLTLRWEPSSQPTIPIVGIIAGLVLFGAMFAGAVVAA
VRWRRKSSDRKGGSYSQAASSDSAQGSDMSLTACKV

A7401 [Patient PP638] 195AA [121AA overlap]

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWD
QETRNVKAHSQTDRVDLGTLRGYYNQSEAGSHTIQMMYGCDVGPDGRLLRGYQQDAYDGKE
LHRLERGPALLDRGGHGGSDHPAQVGGGPCGGAVESLPGGHVRGVAPQIPGEREGDAAAHG
RPQDAYDSPRCL

Alpha 1: GSH…SEA
Alpha 2: GSH…DGK

- Genotype: A*74:01 Mutation [Adenosine Insertion]
- Frameshift caused alteration and early termination
- No Transmembrane Element
- Creation of a cytosolic aberrant protein

Figure 2. A*02:01 was the dominant antibody interaction and completely accounted for A*74:01 cross-reactivity

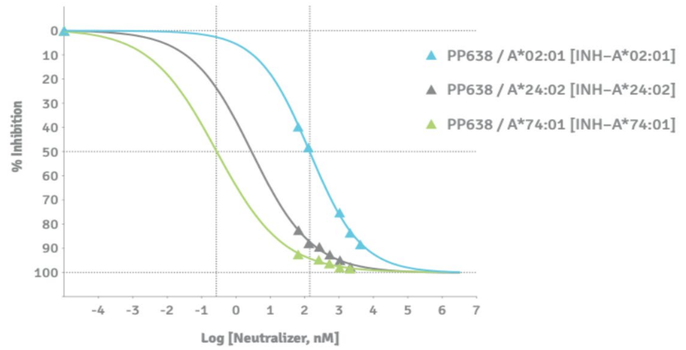


Figure 3. A*74:01 showed preserved binding potency but markedly reduced maximal binding

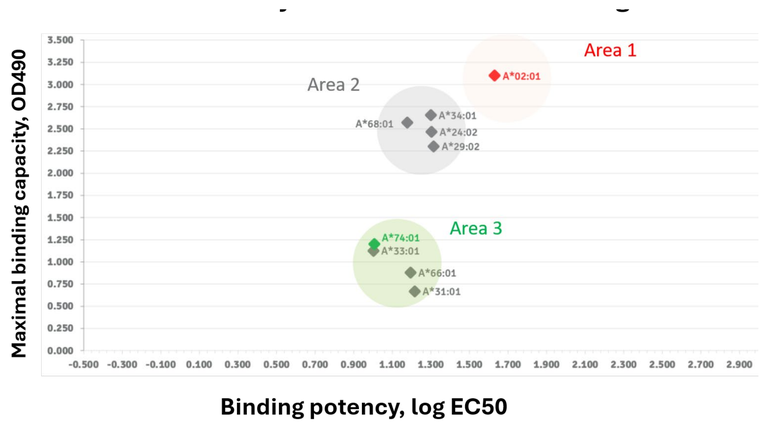


Figure 4. A*02:01 was the dominant antibody interaction and completely accounted for A*74:01 cross-reactivity

A*02:01 Neutralization



Interpretation

MFI-only interpretation	Integrated interpretation
Patient has antibody against self A*74	Patient carries a noncanonical, likely nonexpressed A*74-like allele
Strong A*74 MFI implies strong anti-A*74 antibody	A*74 has measurable potency but limited maximal binding
A*74 may be the immunizing antigen	A*02:01 is the dominant immunizing specificity
Possible autoantibody	Cross-reactive alloantibody recognition
A*74 signal could be treated as an independent specificity	Clinical interpretation should account for shared recognition and allele expression

CONCLUSIONS

MFI correctly detected antibody binding but misrepresented its biological origin and significance. Genetic concordance, expression context, binding capacity, and inhibition profiling established that apparent anti-A**74 self-reactivity reflected A**02:01-driven cross-reactivity against a canonical bead antigen absent from the patient's expressed HLA repertoire.

Disclosures

Jacob Collard and Rico Buchli work for HLA Protein Technologies Inc.