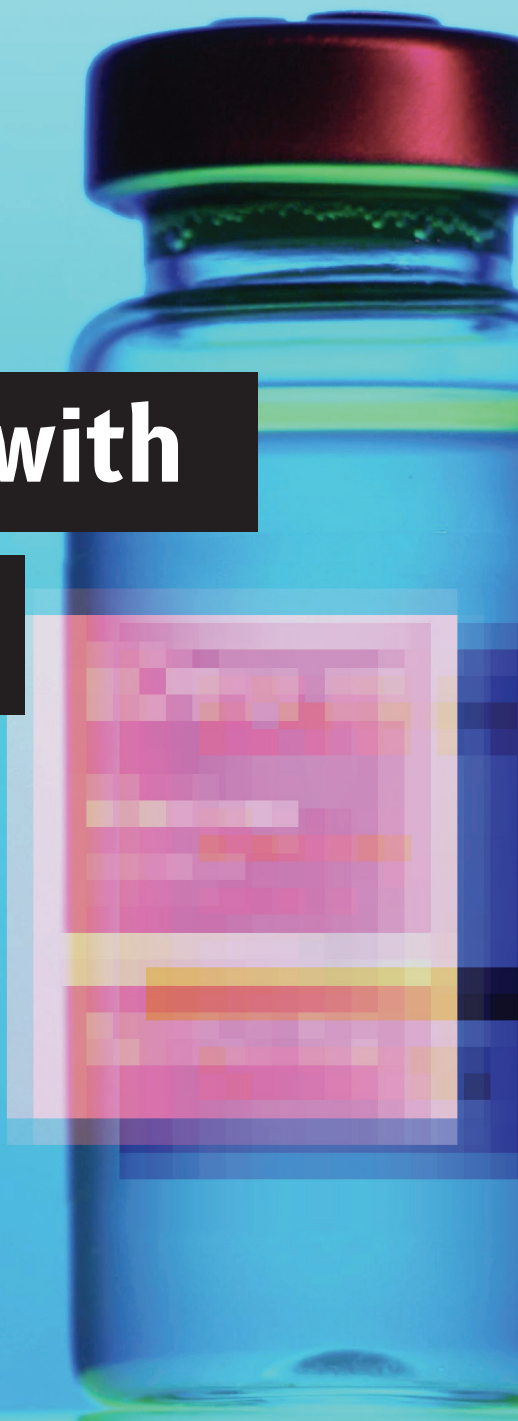
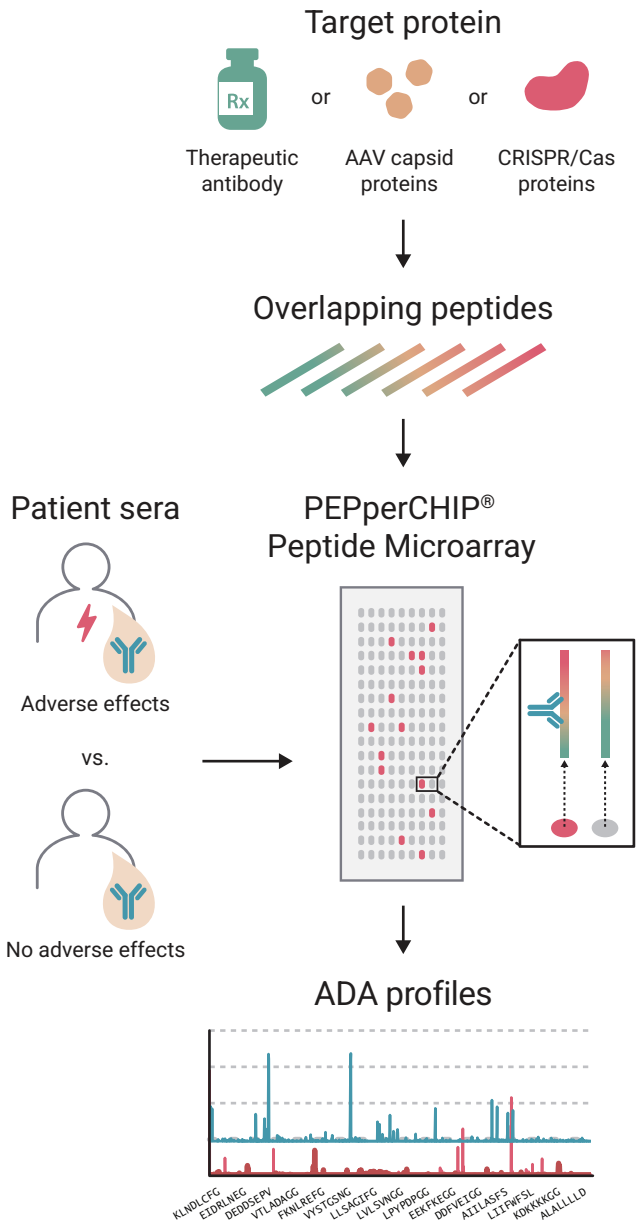
PEPperPRINT
DECIPHERING IMMUNITY

CASE STUDY

Beyond the label: Mapping anti-drug antibody responses with peptide microarrays



We used high-density PEPperCHIP® Peptide Microarrays to detect and profile ADA responses in human sera. Our technology enables rapid, high-resolution analysis of immunogenic epitopes, helping researchers assess treatment risks and optimize drug design.



The Challenge

The rise of therapeutic proteins and gene-editing technologies has transformed modern medicine, but they often come with the risk of immunogenicity. ADAs can reduce drug efficacy and cause adverse effects, making it crucial to understand how patients' immune systems respond to these treatments.

Our Approach

Using our PEPperCHIP® Peptide Microarrays, we screened patient and healthy control sera against overlapping peptides derived from therapeutic and genome editing proteins. This method allowed us to perform different studies where we:

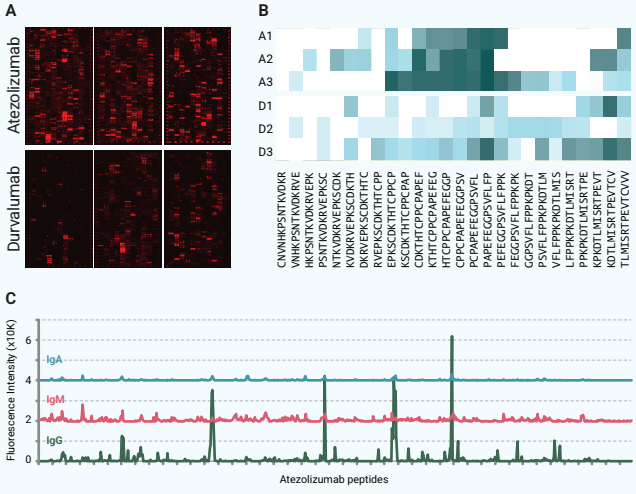
- Compared ADA responses to two PD-L1 inhibitors
- Identified immunogenic epitopes linked to adverse drug effects
- Detected pre-existing immune resposes to CRISPR/Cas proteins

◀ **ADA Epitope Mapping with PEPperCHIP® Peptide Microarrays**. Amino acid sequences of therapeutic antibodies or gene-editing protein components are converted into overlapping peptides and printed onto glass slides. Patient or healthy sera are incubated on the microarray, and ADAs present in the sample bind their corresponding epitopes on the microarray surface. The resulting ADA response profiles can be compared across different samples to identify and monitor pre-existing or treatment-emergent immune responses.

Case 1: A tale of two PD-L1 inhibitors

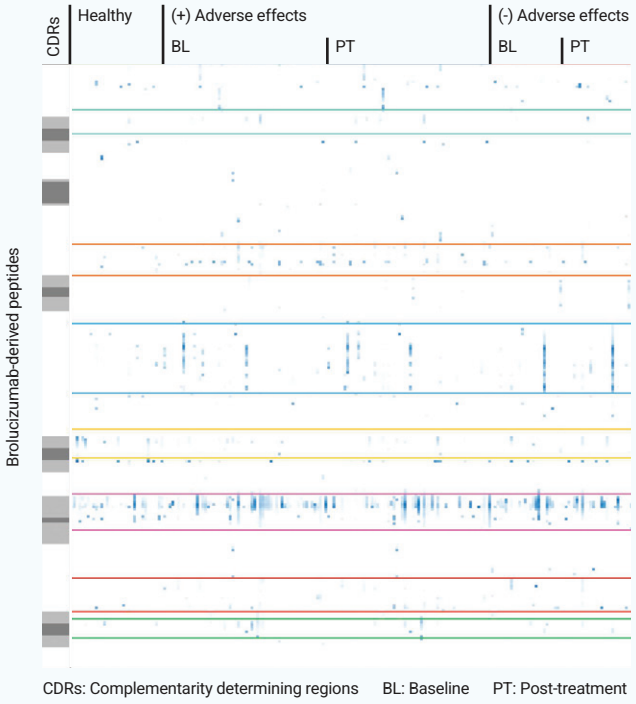
We analyzed serum samples from lung cancer patients treated with two well-characterized PD-L1 inhibitors, Atezolizumab and Durvalumab. Our results showed:

- In accordance with literature, ADA responses were stronger against Atezolizumab
- IgG was the dominant antibody isotype, while IgA and IgM responses were minimal
- Larger cohort studies could further clarify immunogenic differences between the two drugs



ADA Responses in Atezolizumab- or Durvalumab-treated lung cancer patients.(A) Microarray scans, (B) heat map of peptide hits, and (C) intensity profiles for isotype analysis. ▶

Case 2: Searching for clues behind drug's rare adverse effects



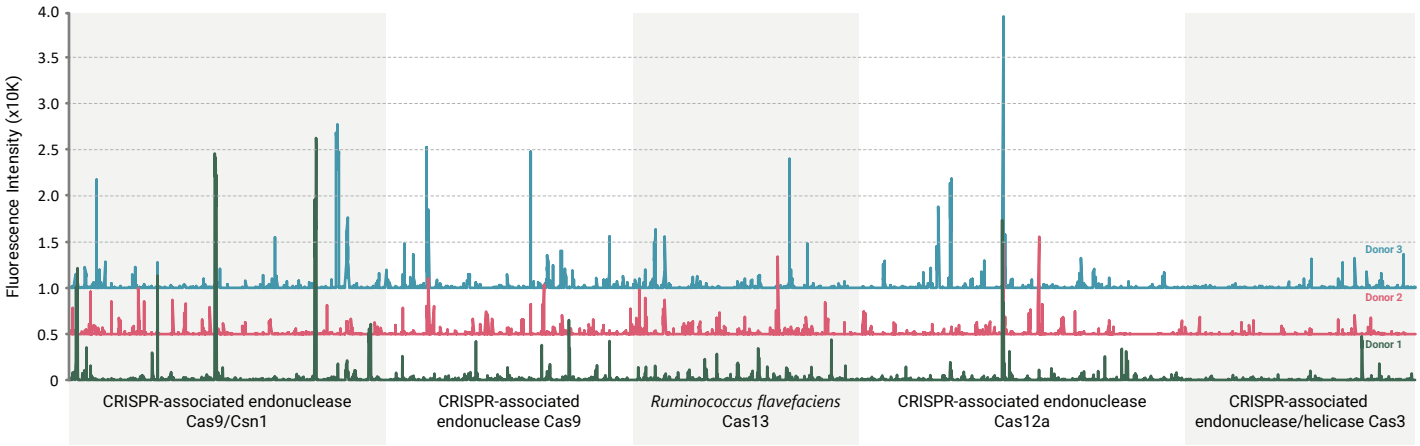
Patients treated with the anti-VEGF antibody Brolucizumab sometimes developed retinal inflammation or vascular occlusion. Our microarray analysis revealed:

- Specific linear epitopes recognized by ADAs in both treated and untreated patients
- Class-switched, high-affinity immune responses indicating a strong adaptive immune reaction linked to ADA epitopes
- Potential for identifying biomarkers to predict patients at risk

◀ **Linear epitope mapping of human IgG responses against a brolucizumab-derived peptide library.** Heat map showing normalized mean fluorescence intensitiy signals corresponding to ADA binding to specific peptides.

Figure modified from Karle, Anette C et al. "Anti-brolucizumab immune response as one prerequisite for rare retinal vasculitis/retinal vascular occlusion adverse events." *Science translational medicine* vol. 15,681 (2023): eabq5241. doi:10.1126/scitranslmed.abq5241

Case 3: Detecting traces of past bacterial encounters



▲ **IgG responses against selected CRISPR/Cas proteins**. Intensity plots showing response profiles against five different CRISPR/Cas effector proteins.

CRISPR-based therapies hold great promise, but immune responses to bacterial Cas proteins can affect their safety and efficacy. We screened and analyzed sera from healthy individuals which showed:

- Pre-existing IgG responses to selected CRISPR/Cas proteins
- Proof-of-concept data that warrants further investigation in larger patient cohorts

Conclusion

PEPperPRINT's peptide microarrays provide a powerful, high-resolution, and high-throughput tool for ADA epitope mapping. By identifying immunogenic sites, our platform helps de-risk biotherapeutics, optimize patient selection, and improve drug design. Whether evaluating the safety of therapeutic antibodies or gene-editing tools, ADA epitope mapping is a key step in immunogenicity risk assessment.

How can we support your research?

Talk to our anti-drug antibody epitope mapping experts

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