Webinar Q&A Report:
De-Risking Immunotherapy Targets with Next-Gen HLA Discovery Platforms

1. **Is the EC50 dependent on the affinity of the control peptide for the HLA?**

   IC50 values are influenced by the affinity of out tracer peptide. However, in the Class II assay I presented, the setup is fundamentally different and avoids that dependency. This Class II assay is not based on fluorescence polarization or a competitive binding format. Instead, it directly measures the formation of HLA–peptide complexes. The peptide of interest is biotinylated and captured on a streptavidin-coated ELISA plate. As we titrate the peptide concentration, increased complex formation leads to a stronger OD490 signal.

   Because this is a direct binding and complex formation assay (rather than a competition assay), the EC50 reflects the intrinsic ability of the peptide to form stable HLA complexes under the assay conditions. In other words, it is not dependent on the affinity of a separate control peptide but rather represents a standardized measure of potency within this system.

   The "landscape" plot I showed summarizes parameters derived from these dose–response curves. Notably, the maximal signal correlates linearly with potency, indicating that both parameters consistently reflect peptide–HLA affinity—higher values corresponding to stronger binding and more stable complex formation.

2. **Do you need HLA-DM in the reaction to load on to the complex?**

   No, we do not. The approach of our exchange program is that we natively create HLA molecules loaded with endogenous peptides. This is our base protein. We use transfectant cell lines from which we harvest the protein, like an antibody. The cells secrete the HLA molecules, which pass through the Golgi, become glycosylated, and are properly folded. We do not perform any biochemical modifications. In the end, we harvest the final product as it would appear on the cell surface. We use affinity purification to obtain high-quality molecules that are more than 95% pure. These molecules are then subjected to a proprietary mechanism that gently opens them and replaces the endogenous peptide with a synthetic peptide. Based on the peptide binding assay, we know this conformation is stable and allows us to produce milligram quantities. While HLA-DM is involved in the natural process, we do not use it to support the exchange.

3. **How many Class 1 alleles do you have access to?**

   This is our biggest advantage. Currently, we have 284 HLA cell lines in our archive. We are not always able to produce all proteins, but at present we have 128 Class I alleles available as

proteins. Out of 157 Class II cell lines, we have produced 90. Many of these are DQ and DP alleles, which we have not yet produced due to lower demand and less common assay technologies. With our repertoire, we aim to cover 90% of the human population. Occasionally, we are unable to provide a specific allele, but there are often alternative alleles that closely match the requirement. Overall, we believe we offer the broadest range of alleles currently available in this field.

4. **How do you compare affinities between different HLA – I assume you use a different control peptide for each HLA and the affinity is always relative this peptide?**

   Comparing affinities between HLA alleles is somewhat challenging, as we use different tracer peptides for each assay. However, we aim to ensure that the affinities of these tracer peptides are similar. There can still be variation, where trends indicate stronger IC50 values—for example, for A02:01 compared to other alleles. We observe these differences particularly between A*03:01 and A*011:01, which bind similar peptide pools and show strong overlap. Because of these variations, it is important to be cautious when stating that one allele is stronger than another, as each assay represents its own system. In general, the differences are not large—typically only a few-fold rather than orders of magnitude. These comparisons are most meaningful when viewed on a logarithmic scale.

5. **Is there any alternative approach to study the epitopes other than in this way to identify the immunogenic and produce immune responses?**

   You're absolutely right—in silico predictions are valuable for narrowing candidates but often come with a high false-positive rate, so experimental validation is essential.

   There are a few complementary approaches:
   - *Quantitative peptide–HLA binding assays:* Measuring binding affinity and stability helps prioritize peptides that are more likely to be biologically relevant and immunogenic. This is the first thing you should do.
   - *Mass spectrometry (immunopeptidomics):* Confirms that a peptide is presented on HLA molecules in real biological samples (e.g., patient tissue or infected cells). This is particularly powerful when you already have candidate epitopes.
   - *T cell–based assays:* Functional validation using tetramer staining, T cell activation assays (e.g., ELISPOT, cytokine release), or cytotoxicity assays to confirm immune recognition.
   - *pHLA-specific antibodies (e.g., TCRm):* Can be used to directly detect and visualize peptide–HLA complexes on cells.
   - *In vivo models (where applicable):* Useful for confirming immunogenicity and immune response in a physiological setting.

   In practice, combining prediction + biochemical validation + functional assays provides the most reliable path to identifying truly immunogenic epitopes.

6. **How can we design antibodies for specific antigens and relate it to our wet lab work? Is there any computational approach?**

   Great question—antibody discovery today for HLA/peptide complexes is typically a wet lab approach first before antibodies are fine-tuned using computational designs.

   - *Experimental (wet lab) approaches:*
     The most reliable methods include:

- Immunization-based discovery (animal or human B cell sourcing)
- Display technologies (phage, yeast) to screen large antibody libraries
- Selection against defined antigens, including peptide–HLA complexes

- *Computational approaches:*
  In silico tools (structure modeling, docking, and AI-based design) can help predict binding regions (epitopes)and guide antibody sequence design or optimization. These are very useful for screening and narrowing candidates but still require experimental confirmation.

- *Link to your work:*
  For epitope-driven applications (like subunit vaccines or pHLA targets), success depends heavily on having a well-defined, validated antigen. High-quality antigen presentation (e.g., correctly folded peptide–HLA complexes) is critical to ensure you generate specific and functional antibodies.

In practice, computational tools help guide and accelerate, but wet lab selection and validation remain essential to obtain high-quality antibodies.

7. Is there a linear correlation with strength of peptide binding with tetramer binding to T lymphocytes?

We recently initiated a project with the NIH in which we received a cell line expressing T-cell receptors recognizing MLANA. This receptor can identify two different peptides: one with medium affinity and one with high affinity. We observe a clear and significant difference, where the tetramer shows a strong signal with the high-affinity peptide, while the response to the medium-affinity peptide is approximately one log lower. The medium-affinity peptide still produces a strong response, but higher affinity clearly enhances tetramer binding. It is also important to consider that not all fluorophores produce the same signal intensity. We recommend specific tetramers to optimize this technology, as some fluorophores simply do not provide sufficient staining. This is not related to the HLA or the tetramer itself, but rather to the properties of the fluorophore.

8. Intrigued about what "good" looks like for validation of NK / Cellular therapeutics using HLA-peptides assays.

“Good” validation is about building confidence across multiple layers, from biochemical accuracy to functional relevance:

- *Defined and high-quality pHLA reagents:*
  Use well-characterized, stable HLA–peptide complexes to ensure you are testing the correct target with high fidelity.

- *Quantitative binding and specificity:*
  Demonstrate strong and selective interaction with the intended peptide–HLA complex, including assessment of potential cross-reactivity.

- *Physiological relevance:*
  Confirm that the target peptide is presented on cells (e.g., via immunopeptidomics or cell-based validation).

- *Functional activity:*

Show that NK or engineered effector cells respond appropriately—such as target cell killing, cytokine release, or activation markers.

- *Safety / selectivity profiling:*
  Evaluate off-target recognition across related peptides or healthy cell contexts to reduce downstream risk.

In short, strong validation connects accurate target definition → specific recognition → functional response, all in a biologically relevant context.

Further, NK cell biology is strongly linked to HLA-C, as it is one of the primary ligands for KIR receptors that regulate NK cell activity (inhibition vs. activation). In this context, validation often focuses on specific HLA-C binding.

9. How you see the future of HLA matchmaking and managing its impact in transplantation?

HLA matchmaking will remain central to transplantation success, as it directly impacts:

- Graft acceptance and long-term survival
- Risk of rejection and graft-versus-host responses
- Need for immunosuppression and associated complications

What is becoming increasingly clear, however, is that matching at the allele level is not always sufficient. The field is moving toward a more refined understanding of:

- Epitope-level matching (eplet-based approaches)
- The fact that the same epitope can behave differently depending on the presenting HLA allele
- Differences in binding affinity and presentation stability, which can influence immunogenicity

This suggests that future strategies will require more precise, quantitative characterization of HLA–peptide interactions, beyond "HLA matchmaker algorithm" rather than relying solely on classical matching approaches.

Overall, I strongly believe in the importance of HLA matchmaking—but its future lies in fine-tuning at the epitope and functional level, enabling more personalized and predictive transplantation strategies.

10. How scalable is the mapping workflow, and how easily can it be integrated into existing discovery pipelines without creating bottlenecks?

The mapping workflow begins with a peptide library and is easy to scale. Every two days, an additional allele can be screened. We currently operate more than 50 assays in which libraries can be tested. The primary bottleneck lies in peptide synthesis rather than in the technology itself. Once the peptides are available, the mapping workflow proceeds smoothly.

11. How do you approach peptide discovery and validation for less common HLA alleles where data and tools are extremely limited?

We have a strong foundation, with over 50 peptide binding assays developed over the past 25 years, which cover the most common alleles. For less common alleles of interest, we have the

capability to develop new assays. For example, we recently developed an assay for HLA-E for a specific customer. The key is identifying a suitable tracer peptide for use in a competition assay. If the protein is already available, converting it into a new assay is straightforward. In cases where the allele is not available, we can generate transfectants and build assays from the ground up. Creating a new cell line takes approximately 1–2 months, followed by several additional weeks for protein production and assay development. Within 2–3 months, we can deliver assays that are not currently available.

12. **Do you assess peptide–HLA binding kinetics (on/off rates) in addition to equilibrium affinity, and how do these parameters influence target prioritization?**

    We no longer focus on binding kinetics. A publication from 2005 demonstrated that on/off rates are not the primary determinant of stable immune complex formation. Instead, we assess stability directly. We observe that these complexes stabilize after approximately three days. In key experiments, we conducted forced degradation studies using heat, showing that Class I molecules remain stable up to 40°C, while Class II molecules are stable up to 60°C. These complexes are highly stable, and our HLA molecules closely resemble native molecules. This aligns with physiological expectations—for example, during fever, peptides do not dissociate from Class I molecules when T cells are most needed. Based on our data, we do not believe that kinetics significantly influences target prioritization.

Contact Details

If you have additional questions for Dr. Rico Buchli or HLA Protein Technologies regarding content from their webinar, please contact them by phone or email:

Rico Buchli, PhD
Chief Scientific Officer
HLA Protein Technologies

HLA Protein Technologies Inc.
Address: 655 Research Parkway, Suite 556, Oklahoma, United States, 73104
Website: https://www.hlaprotein.com/
Email: support@hlaprotein.com
Phone: 405-271-3838

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