Optimizing the Donor Matching Process in Solid Organ Transplantation

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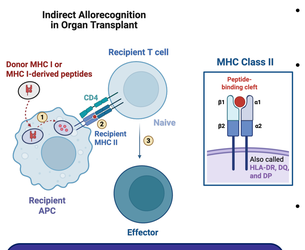
Abstract & Aim

Transplantation is the sole or preferred treatment option for patients with kidney, liver, pancreatic, lung, or heart diseases. Particularly, for kidney transplants, appropriate measures are necessary to optimize the donor matching process. Alloantigen from the donor can be presented by the Major Histocompatibility Complex (MHC) on the recipient's antigen-presenting cells (APCs), triggering adversary antibody and inflammatory responses. Many current prediction methods, however, do not consider the interplay of biologically relevant processes, such as antigen processing by lysosomal proteases, chaperone HLA-DM, or HLA expression level. To improve organ transplant outcomes, we are employing both computational approaches and experimental validation to develop a comprehensive approach to predict the presentation of the donor's MHC-derived alloantigen peptides by the host's MHC class II.

As a proof of concept, the goal of this work is to **develop a systematic approach method** to predict presentations of the **donor's mismatched MHC class I-derived peptides** on **host MHC class II** and their impacts on delayed allograft recognition, paving the way for improving the outcome of organ transplants.

Introduction: MHC II & the Indirect Allorecognition Pathway

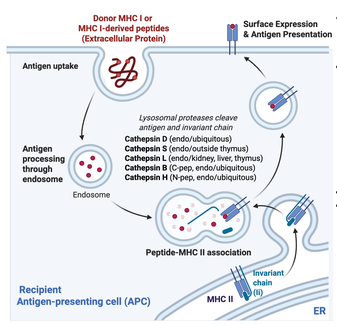


- Among the biggest challenges with transplantation is graft rejection due to **alloimmune responses**
- While the direct allorecognition pathway often result in short-lived acute rejection in the early phase post-transplantation, the **indirect pathway** does not manifest until later, thereby orchestrating the generation of CD4+ T cells, alloreactive B cells, Donor Specific anti-HLA Antibodies (DSA), and unpredictable chronic rejection
- There is a need to improve the **matching process** for organ transplant to improve patient outcome and care (*e.g.* post-transplant immunosuppressant complications)
- The **Major Histocompatibility Complex class II (MHC II)** or **HLA II** plays a central role in the recipient's immune response to a transplant.

As one possible pathway, recipient MHC II molecule can present peptides fragmented from the mismatched MHC I molecules from the donor.

Duneton C., *et al. Nat Rev Nephrol.* **2022**, *18* (10), 663-676.

Introduction: Antigen Processing with Cathepsins



- **Cathepsins** are enzymes known to be essential in antigen processing for the MHC II pathway.
- For the process of peptide generation for loading on MHC class II *in vivo*, the cathepsins below have been identified to be essential, and many of them have overlapping properties (*e.g.* cleavage mode) and functions (*e.g.* break down big peptides; trimming).

Cathepsins considered in the literature

- **Cell-free system**: Cts H, **Cts S**, and Cts B
- **PBMC crude method**: **Cts D**, Cts K, **Cts S**, and **Cts B**

We consider cathepsins D, S, L, B for coverage of cleavage properties and roles, and the availability of the computational tool for cleavage prediction by these proteases.

Honey, K.; Rudensky, A. Y. *Nat Rev Immunol* **2003**, *3* (6), 472–482.
Chapman, H. A.; Riese, R. J.; Shi, G.-P. *Annual Review of Physiology* **1997**, *59* (Volume 59, 1997), 63–88.

Our Comprehensive Approach

Herein, we're employing both computational and experimental validations to predict and evaluate the pools of HLA I-derived mismatched peptides that can be presented by HLA Class II.

- "PIRCHE-II are theoretically predicted epitopes. ….therefore, some of the PIRCHE-II that are predicted to bind to HLA class II molecules, may not be generated biologically by the antigen processing pathway (not **physiologically relevant**). As such, we may overestimate the genuine number of PIRCHE-II for certain donor-recipient couples."
- "Since individual HLA loci and even different HLA alleles within a certain HLA locus may be expressed differently, it is expected that lower expressed HLA alleles are less likely to be recognized by T cells. "
- "It is also expected that PIRCHE-II presented by lower expressed HLA class II alleles are less likely to be recognized by T cells. "
- "The immunological impact of PIRCHE-II is also determined by the HLA-peptide-TCR. Differential engagement of the TCR depending on amino acid residues of the bound peptide on the class II (De Oliveira et al. 2000; Rudolph et al. 2006)."

A Comprehensive Approach for Risk Assessment

HLA-derived Allopeptide Repertoire

Identification of peptides with mismatched amino acids

- **Computational Approaches:** All inclusive vs. cathepsins generated peptides
- **Experimental:** *in vitro* cell-free cathepsin assays; PBMC-derived endosomal, lysosomal assays

HLA Heterozygosity

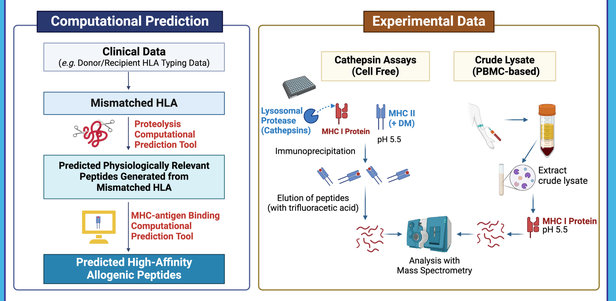
Differential Expression of HLAs

Presentation of Peptides by HLA Class II DR and DQ Molecules

T cell Repertoire

Geneugelijk, K., and Spierings, E. *Immunogenetics* **2020** *72*, 119–129.

Methods: Computational & Experimental Approaches

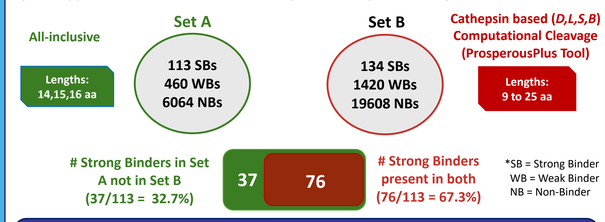


Methods: Computational Analysis

- From our center's past transplant cases, 16 pairs are identified all of which are HLA-DRB1*11:01
- Identify mismatched peptides using **two** alternative approaches:
  - A. Around the mismatched position of HLA Class I identify all the 14-, 15-, 16-amino acid peptides that include the mismatched amino acids and differ from each other by shifting one position at a time **(All-inclusive method)**
  - B. Use *enzymatic cleavage* (cathepsins D, L, S, B) and generate computationally predicted (ProsperousPlus tool) and potentially more physiologically relevant peptides that include the mismatched epitope **(Cathepsin-based computational cleavage)**
- Both sets of 16 are assessed for binding affinity prediction with the same program (*NetMHCIIpan-4.3*)

Results: Computational Prediction

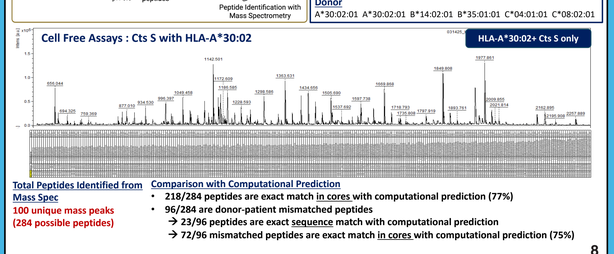
Mismatched peptides of Class I from 16 donor-recipient pairs generated computationally by two approaches (All-inclusive and Cathepsin-based) presented by DRB1*11:01



Among the computationally (All-inclusive method) generated peptides, 67.3% of these peptides were also identified by the computational Cathepsin-based method, while 32.7% were not found to be generated by the Cathepsin-based digestion. It is possible that the physiologically relevant peptides may be less than the computationally generated using the "all inclusive" method used also in PIRCHE.

Results: HLA-A*30:02 Processed by Cathepsin S Mass Spectrometry Analysis

Comparison of Computational Prediction and Experimental Data from Cell Free Assay



Donor HLA Class II DRB1*03:01:01G | **DRB1*11:01:01G**

Donor HLA Class I Mismatches: **A*30:02** | B*14:02 | C*08:02

Patient (P-062)
A*01:01:01 A*03:01:01 B*08:01:01 B*35:01:01 C*04:01:01 C*07:01:01

Donor
A*30:02:01 A*30:02:01 B*14:02:01 B*35:01:01 C*04:01:01 C*08:02:01

Total Peptides Identified from Mass Spec
100 unique mass peaks (284 possible peptides)

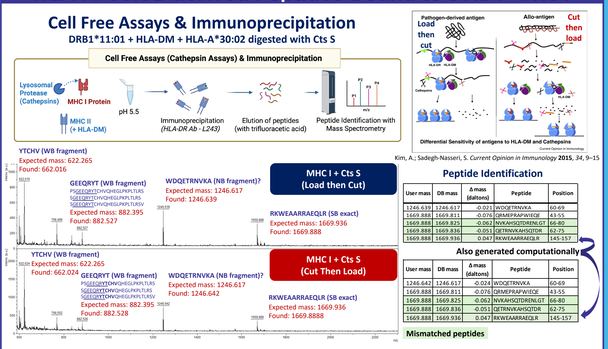
Comparison with Computational Prediction

- **218/284 peptides are exact match in cores with computational prediction (77%)**
- **96/284 are donor-patient mismatched peptides**
  - → **23/96 peptides are exact sequence match with computational prediction**
  - → **72/96 mismatched peptides are exact match in cores with computational prediction (75%)**

Results: Mass Spectrometry Analysis of HLA-A*30:02-derived Peptides Bound to DRB1*11:01

Cell Free Assays & Immunoprecipitation

DRB1*11:01 + HLA-DM + HLA-A*30:02 digested with Cts S



Peptide Identification

User mass	DB mass	Δ mass (daltons)	Peptide	Position
1246.639	1246.617	-0.021	WDQETRNVKA	60-69
1669.888	1669.811	-0.076	QRMEPRAPWIEQE	43-55
1669.888	1669.825	-0.062	NVKAHSQTDRENLGT	66-80
1669.888	1669.816	-0.051	QETRNVKAHSQTDR	62-75
1669.888	1669.936	0.047	RKWEAARREAQLR	145-157

Also generated computationally

User mass	DB mass	Δ mass (daltons)	Peptide	Position
1246.642	1246.617	-0.024	WDQETRNVKA	60-69
1669.888	1669.811	-0.076	QRMEPRAPWIEQE	43-55
1669.888	1669.825	-0.062	NVKAHSQTDRENLGT	66-80
1669.888	1669.816	-0.051	QETRNVKAHSQTDR	62-75
1669.888	1669.936	0.047	RKWEAARREAQLR	145-157

Mismatched peptides

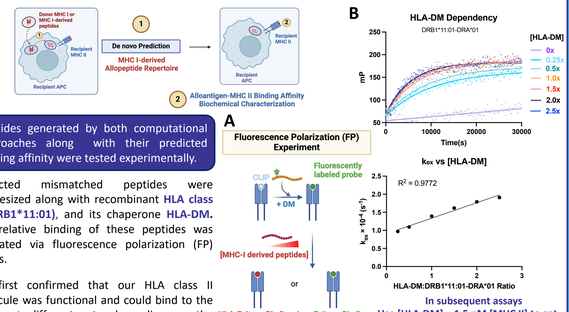
Results: Peptides Generated by Cathepsin: Computational vs. Experimental Data

Computational prediction revealed mismatched peptides bound to DRB1*11:01 as follows

- 27 **strong binders**, with a total of **1 unique core**;
- 30 **weak binders**, with a total of **4 unique cores**;
- 279 **non-binders**, with a total of **55 unique cores**.
- Experimental data showed one donor-patient mismatched peptide binding to DRB1*11:01.
- This peptide matches our computational prediction, is identified as a strong binder, and is among the 5-6 peptides with the highest binding affinity (nM).
- Computational analysis predicted additional peptides, but all share the same peptide core.

MHC	Peptide	Core	Identity	Score_EL	%Rank_EL	Score_BA	%Rank_BA	Affinity (nM)	Bind Level
DRB1_1101	QRKWEAARREAQ	WEAARREAQ	Sequence	0.941072	0.38	0.458186	11.85	301.54	SB
DRB1_1101	QRKWEAARREAQL	WEAARREAQ	Sequence	0.986617	0.1	0.475269	10.29	292.21	SB
DRB1_1101	QRKWEAARREAQLRAYLE	WEAARREAQ	Sequence	0.96676	0.25	0.476166	10.21	289.39	SB
DRB1_1101	AQITQRKWEAARREAQLR	WEAARREAQ	Sequence	0.967344	0.24	0.476521	10.17	287.97	SB
DRB1_1101	RKWEAARREAQLR	WEAARREAQ	Sequence	0.979826	0.15	0.47678	10.16	287.53	SB
DRB1_1101	RKWEAARREAQL	WEAARREAQ	Sequence	0.953332	0.32	0.504326	8.03	213.38	SB
DRB1_1101	QRKWEAARREAQLRAYLEG	WEAARREAQ	Sequence	0.931502	0.43	0.526939	6.6	167.07	SB
DRB1_1101	AQITQRKWEAARREAQLRA	WEAARREAQ	Sequence	0.971617	0.22	0.545617	5.53	136.5	SB
DRB1_1101	AQITQRKWEAARREAQL	WEAARREAQ	Sequence	0.938632	0.41	0.555961	5.02	122.05	SB
DRB1_1101	RKWEAARREAQLRA	WEAARREAQ	Sequence	0.983827	0.12	0.564132	4.62	111.72	SB
DRB1_1101	RKWEAARREAQLRAYLE	WEAARREAQ	Sequence	0.903075	0.56	0.568072	4.44	107.06	SB
DRB1_1101	AAQMAAQTQRKWEAARREAQLR	WEAARREAQ	Sequence	0.825527	0.95	0.574382	4.18	99.99	SB
DRB1_1101	QRKWEAARREAQLR	WEAARREAQ	Sequence	0.993053	0.05	0.574696	4.17	99.65	SB
DRB1_1101	RKWEAARREAQLRAYL	WEAARREAQ	Sequence	0.95362	0.32	0.581136	3.91	92.95	SB
DRB1_1101	QRKWEAARREAQLRAYLEGT	WEAARREAQ	Sequence	0.861051	0.77	0.581886	3.88	92.19	SB
DRB1_1101	AQITQRKWEAARREAQLRAYL	WEAARREAQ	Sequence	0.917576	0.49	0.59147	3.47	81.33	SB
DRB1_1101	AQITQRKWEAARREAQLRAYLE	WEAARREAQ	Sequence	0.92371	0.46	0.595848	3.39	79.27	SB
DRB1_1101	AQITQRKWEAARREAQLRAYLEG	WEAARREAQ	Sequence	0.927983	0.44	0.597494	3.34	77.87	SB
DRB1_1101	AQITQRKWEAARREAQLRAYLEGT	WEAARREAQ	Sequence	0.933133	0.43	0.598794	3.29	76.78	SB
DRB1_1101	QRKWEAARREAQLRAYL	WEAARREAQ	Sequence	0.983203	0.12	0.599404	3.27	76.28	SB
DRB1_1101	AAQMAAQTQRKWEAARREAQLRA	WEAARREAQ	Sequence	0.933184	0.42	0.599492	3.27	76.2	SB
DRB1_1101	AQITQRKWEAARREAQLRAYLEGTC	WEAARREAQ	Sequence	0.933548	0.42	0.599801	3.26	75.95	SB
DRB1_1101	AQITQRKWEAARREAQLRAY	WEAARREAQ	Sequence	0.953116	0.33	0.601605	3.2	74.48	SB
DRB1_1101	AADMAAQTQRKWEAARREAQLRAY	WEAARREAQ	Sequence	0.943679	0.37	0.603341	3.14	73.1	SB
DRB1_1101	QRKWEAARREAQLRAYL	WEAARREAQ	Sequence	0.995316	0.06	0.613771	2.81	65.29	SB
DRB1_1101	RKWEAARREAQLRAY	WEAARREAQ	Sequence	0.975457	0.19	0.637359	2.32	50.59	SB
DRB1_1101	QRKWEAARREAQLRA	WEAARREAQ	Sequence	0.994999	0.03	0.654424	1.68	42.06	SB

Methods: Assessment of Peptide Binding Affinity



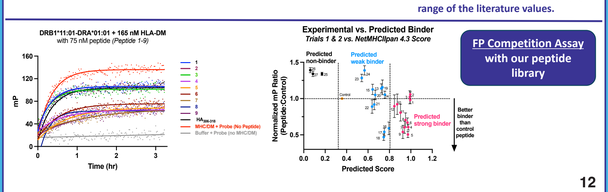
Peptides generated by both computational approaches along with their predicted binding affinity were tested experimentally.

Predicted mismatched peptides were synthesized along with recombinant **HLA class II (DRB1*11:01)**, and its chaperone **HLA-DM**. The relative binding of these peptides was evaluated via fluorescence polarization (FP) assays.

We first confirmed that our HLA class II molecule was functional and could bind to the probe at different rate depending on the amount of the chaperone HLA-DM.

In subsequent assays Use [HLA-DM] = 1.5 nM [MHC II] to get good FP signal

Results: Assessment of Peptide Binding Affinity



FP Competition Assay with a known epitopic peptide

PKYVKQNTLKLAT is a linear peptidic epitope (**HA 306**; epitope ID 48237) studied as part of Hemagglutinin from Influenza A virus. It is a known binder for DRB1*11:01 (IEDB Database)

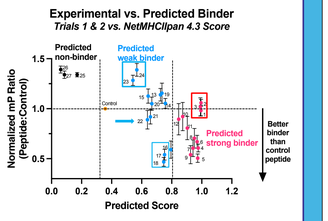
Published literature IC_{50} value: 126 nM (John Sidney; IEDB Submission 2017); 600 nM (C de Lalla J Immunol 1999)

→ **Our IC50 value matched with the range of the literature values.**

FP Competition Assay with our peptide library

Results: Peptide Binding to HLA II Computational vs. Experimental Data

Peptide ID	Peptide Sequence	Peptide Core	Peptide Length	Predicted Score	Predicted IC50 (nM)
1	TQRKWEAARREAQLRA	WEAARREAQ	16	0.994015	63.4
2	QRKWEAARREAQLRA	WEAARREAQ	15	0.994999	42.1
3	QRKWEAARREAQLRAYL	WEAARREAQ	16	0.993316	65.3
4	MRYFYTAVSRPGRGEP	FYTAVSRPGR	16	0.976575	23.3
5	MRYFYTAVSRPGRGEZ	FYTAVSRPG	15	0.973325	10.4
6	RYFYTAVSRPGRGEP	YTAVSRPGR	15	0.969325	14.1
7	SMKYFFTSVSRPGRGE	FFTSVSRPG	16	0.941833	21.3
8	MKYFFTSVSRPGRGE	FFTSVSRPG	15	0.950956	10.3
9	MKYFFTSVSRPGRG	FFTSVSRPG	14	0.925863	27.1
10	MRYFHTSVSRPGRGEP	FHTSVSRPG	16	0.873241	55.0
11	MRYFHTSVSRPGRGE	FHTSVSRPG	15	0.903428	19.0
12	RYFHTSVSRPGRGE	FHTSVSRPG	14	0.846437	96.4
13	TKRKWEAAHAAEQDRA	WEAAHAAEQ	16	0.72491	1035.5
14	KRKWEAAHAAEQDRA	WEAAHAAEQ	15	0.757907	876.4
15	KRKWEAAHAAEQDR	WEAAHAAEQ	14	0.643224	2060.7
16	SMRYFHTAMSRPGRGE	FHTAMSRPG	16	0.794697	45.0
17	SMRYFHTAMSRPGRG	FHTAMSRPG	15	0.752524	15.7
18	MRYFHTAMSRPGRG	FHTAMSRPG	14	0.747223	59.5
19	SMRYFTSVSRPDSGE	FTSVSRPDS	16	0.735242	85.4
20	SMRYFSTSVSRPDSG	FSTSVSRPD	15	0.667726	33.1
21	MRYFSTSVSRPDSG	FSTSVSRPD	14	0.658649	124.6
22	SDGRFLRGYRQDAYDQ	FLRGYRQDA	16	0.638666	503.9
23	SDGRFLRGYRQDAYD	FLRGYRQDA	15	0.541845	216.4
24	DGRFLRGYRQDAYD	FLRGYRQDA	14	0.547451	582.9
25	QITQRKWEAAREAEQ	WEAAREAEQ	16	0.17059	2923.9
26	QITQRKWEAAREAEQ	WEAAREAEQ	15	0.061055	3005.8
27	ITQRKWEAAREAEQ	WEAAREAEQ	14	0.085353	6094.8
Control*	PKYVKQNTLKLAT	YVKQNTLKL	13	0.357847	705.0



FP assay demonstrated that for 56% of the peptides, the relative binding strength were concordant with the predicted categorization as being strong, weak, or non-binders.

Conclusion

Prediction of Physiological Relevant Peptides Generated from Mismatched HLA

- We proposed **two** computational methods:
  (1) All-inclusive method; (2) Cathepsin-based computational cleavage (enzymatic approach)
- For our example, among the computationally (All-inclusive method) generated peptides, 67.3% of these peptides were also identified by the computational Cathepsin-based method, while 32.7% were not found to be generated by the Cathepsin-based digestion. **It is possible that the physiologically relevant peptides may be less than the computationally generated using the "all inclusive" method used also in PIRCHE.**
- As a proof of concept, experimentally via a cell-free assay and mass spectrometry, we digested HLA-A*30:02 with cathepsin S, showing that **many experimentally produced peptide fragments matched either as exact sequences or exact cores.**
- We further assessed **binding** of the HLA-A*30:02 derived peptides (from cathepsin S digestion) with the donor's mismatched HLA class II (DRB1*11:01) using immunoprecipitation followed by mass spectrometry. **We found peptides that were exact match with computationally predicted strong binder peptides, and some peptides that partially matched with predicted peptides.**

Prediction of Binding Affinity to HLA Class II

- Some peptides generated by both computational approaches were assessed experimentally.
- We successfully set up a fluorescence polarization (FP) assay system to validate the binding affinity of our peptides to DRB1*11:01. FP assays demonstrated that at least 56% of the peptides predicted binding had relative binding strength consistent with computational predictions.

Overall

Our experimental data suggested that computational approaches have merit, yet they require further optimization.

Future Directions

- Conduct both **cell-free** and **PBMC crude lysate assays** with biological samples, coupled with **immunoprecipitation** and **proteomic studies**, to further benchmark the computational prediction.
- Binding studies with **different HLA class II**: DRB1*11:01; DRB1*07:01; DRB1*15:01.
- To properly assess the role of HLA mismatched epitopes in the context of T-cell responses there will be a need to identify **what peptides of the many possible mismatched are to be accounted for**.
- Experimental approaches assessing peptide binding demonstrate that while the binding assay itself is reproducible and credible, **the computational assessments need further optimization**. Be aware of DR, DQ differences in terms of peptide binding.
- **Should we be concerned with quantitative issues?** (allele specific differential expression of HLA antigens and promoter-enhancer polymorphisms of HLA genes). In the context of transplantation, we need to further explore the differential expression of HLAs on relevant tissues and assess the role of this differential expression in transplantation.

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