

Identification of *Mycobacterium avium* Peptides Presented by Major Histocompatibility Complex Through Immunopeptidomics

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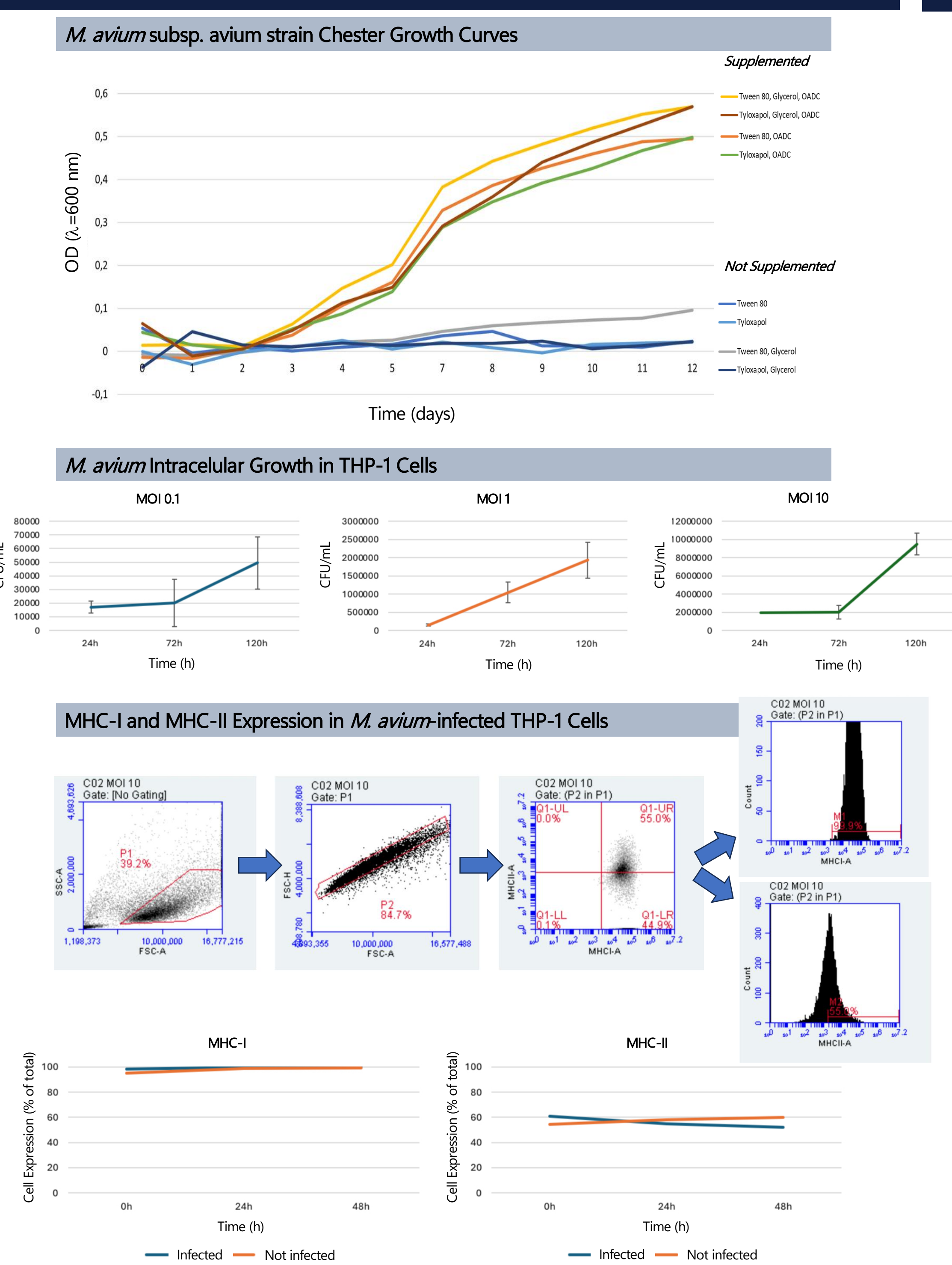
Introduction

- M. avium* is a slow growing Nontuberculous Mycobacteria (NTM)
- Opportunistic intracellular pathogen, affecting the lungs of immunocompromised patients
- M. avium* infections can be dramatic in patients with co-morbidities
- Treatments are available with modest therapeutic efficacy, but no vaccine

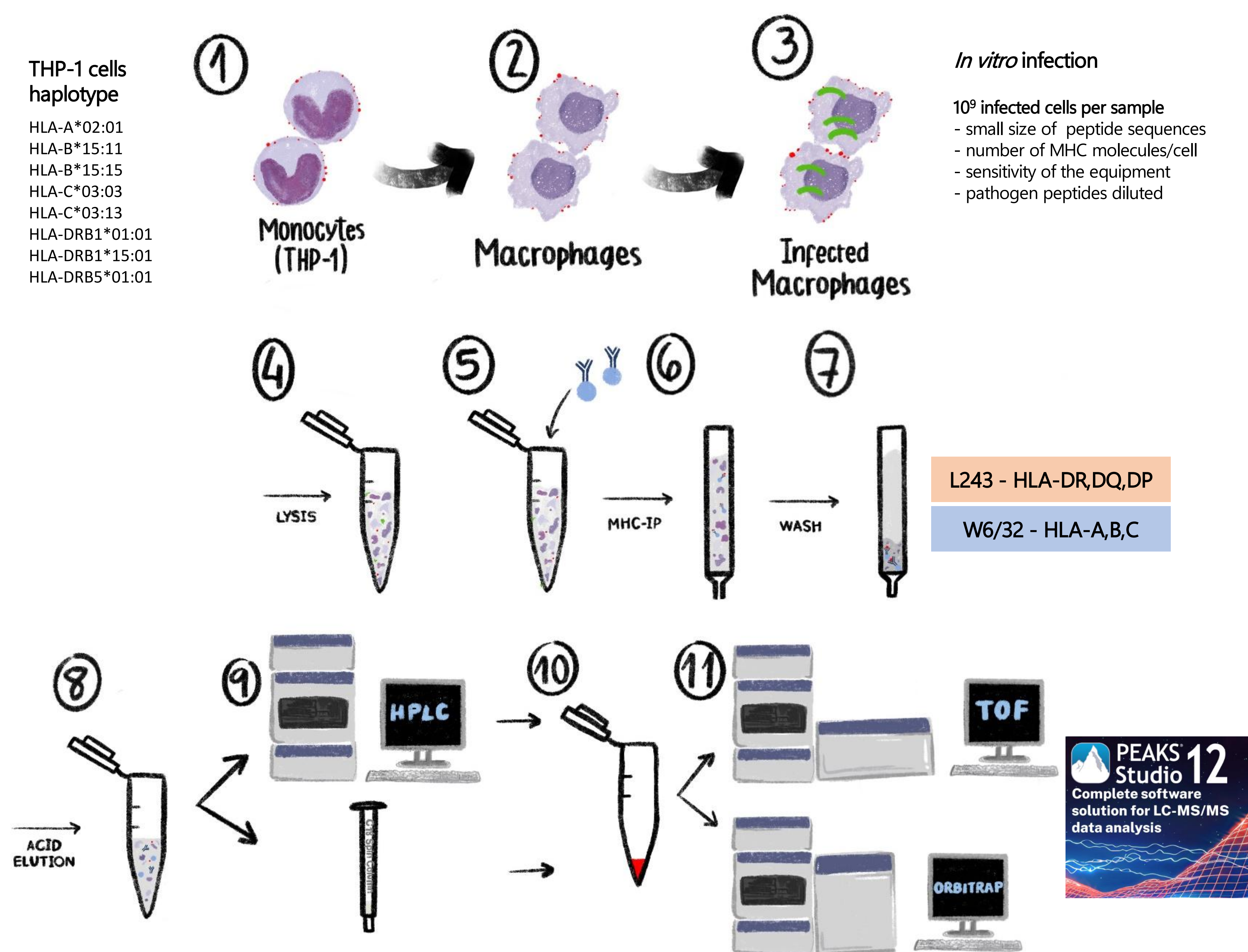
Goals

- Identification of peptides presented by MHC-I and MHC-II in *M. avium*-infected macrophages
- Discovery of antigens for the development of a new vaccine candidates

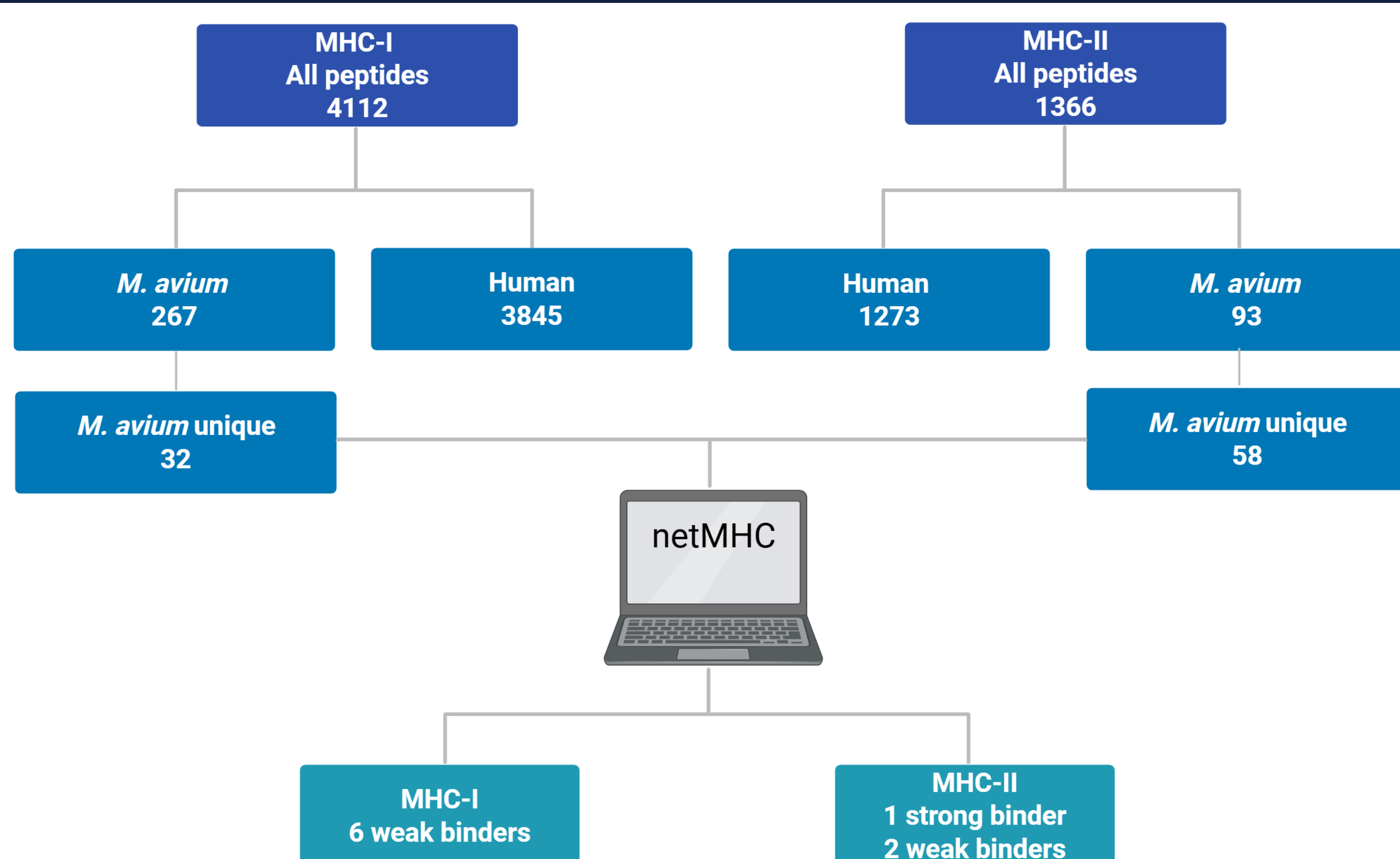
THP-1-*M. avium* Interaction



Immunopeptidomics experimental pipeline



Peptide identification



Antigen Identification

Table 1: MHC-I Peptide Identification

Peptide	Protein	Assession	n	netMHC Best Binder
LLLIIVLVV	DUF4333 domain-containing protein	UPI000392778F	9	HLA-A*02:01 (1.028)
ALLLIIVLV			9	HLA-A*02:01 (1.445)
ALALAGVSA	ABC transporter ATP-binding protein	UPI0001B5A42D	9	HLA-A*02:01 (1.610)
LLLGATPL	Ribosomal RNA small subunit methyltransferase I	UPI00002382AD	8	HLA-A*02:01 (2.354)
LPVGAAAA	Hypothetical protein	UPI0001B59EC9	9	HLA-B*15:11 (2.640)
VRADSVAGY	MMPL family transporter	UPI000314B95D	9	HLA-B*15:11 (2.789)

MHC-I peptides with a rank ≤ 0.5 or between 0.5 and 3.0 were considered strong or weak binders, respectively.

Table 2: MHC-II Peptide Identification

Peptide	Protein	Assession	n	netMHC Best Binder
NNPVLIGEPGVGKTA	Chaperone protein ClpB/	UPI0000E84826/	15	HLA-DRB5*01:01 (1.18)
NNPVLIGEPGVGKT	ATP-dependent Clp protease ATP-binding subunit	UPI00044566FC	14	HLA-DRB5*01:01 (2.30)
PVAISAGMATLVI	ABC transporter permease	UPI0001B59C21	13	HLA-DRB1*01:01 (8.15)

MHC-II peptides with a rank ≤ 2 or between 2 and 10 were considered strong or weak binders, respectively.

Discussion

- THP-1 macrophages infected with *M. avium* express MHC-I and MHC-II
- We have identified 32 MHC-I and 58 MHC-II *M. avium* peptides, with a Peaks Score higher than 15 (equivalent to a p-value < 0.5)
- Using netMHCpan, we shortlisted 6 MHC-I and 3 MHC-II binder peptides
- Strikingly 2 sets of peptides are nested, a typical feature of MHC-bound peptides
- Combining Peaks Score hierarchization with peptide binding affinity, we shortlisted a number of peptides currently being evaluated for its capacity to stimulate T-cell responses

Future perspectives

- Selection of the most immunogenic peptides by ELISpot
- Design new mRNA vaccine candidates encoding *M. avium* antigens
- Validation of vaccine candidates in a murine *M. avium* challenge model

References

- Bettencourt et al. NPJ Vaccines. 2020.
- Barbosa & Bettencourt, in Vaccinology and methods in vaccine research. Academic Press, 2022.
- Almujri et al. *In press*.
- Mateus et al. Unpublished.