MyT™ platform for unbiased discovery of most abundant and immunogenic epitopes

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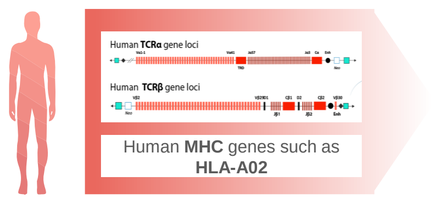
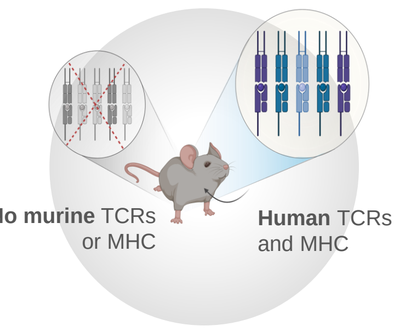
Background

Targeting optimal HLA-presented epitopes of tumor antigens is key for effective therapy with T-cell receptor-engineered T-cells (TCR-Ts). Epitopes must be well processed (leading to high cell surface abundance), as well as immunogenic (allowing for the effective activation of T-cells). Here, we demonstrate that T-knife's MyT platform can identify such relevant T-cell epitopes across a range of HLAs. The MyT platform is a transgenic mouse model that expresses the entire human TCR alpha /beta gene loci along and seven common human class-I HLAs. Due to lack of central immunological tolerance against human antigens in the mice, immunization with human tumor antigens, most commonly self-antigens, induces strong T-cell activation and expansion of T-cell clones with fully-human, high-affinity TCRs. Importantly, by immunizing mice with full-length antigens, we can induce unbiased T-cell responses and identify epitopes which are optimal targets for TCR-T therapy.

MyT platform





MyT platform mice carry the entire human TCRα/β gene loci and single or multiple HLA class-I alleles, covering approximately 80% of the HLA types found in the United States and Europe. They are engineered to lack expression of murine TCRα, TCRβ and MHC.

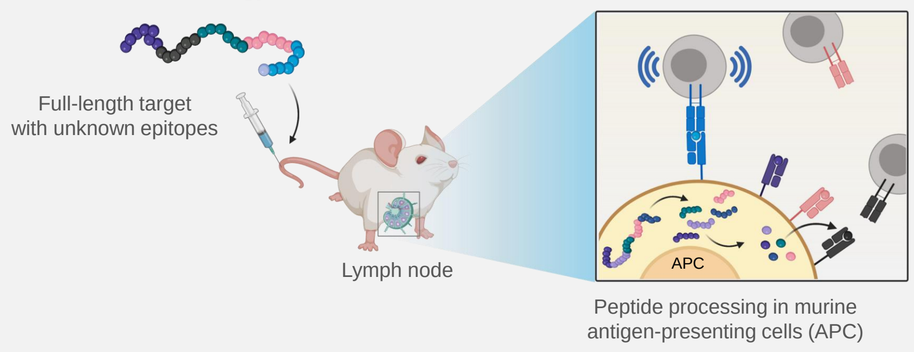
Methods

We compared the antigen processing and presentation in the MyT platform versus that in human tumor cells via immunopeptidomics to verify the suitability of the MyT platform for epitope discovery for TCR-T therapy. We then compared epitope abundance detected by immunopeptidomics to the immunogenicity of the epitopes in the MyT platform after full-length antigen immunization to analyze the ability of the MyT platform to select epitopes of high relevance for TCR-T therapy.

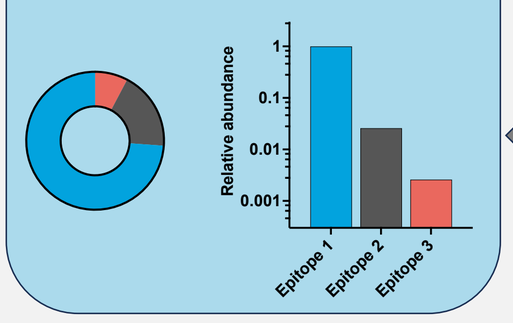
Epitope ranking by MyT platform immunogenicity

1. Epitope-unbiased immunization strategy

2. Selection of optimal epitope/HLA combination by natural immune response



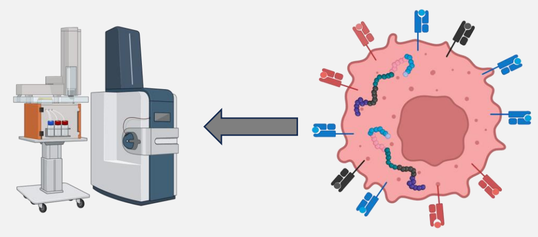
3. Selection of optimal epitopes for TCR-T therapy by ranking



Epitope ranking by immunopeptidomics

2. Epitope quantification by immunopeptidomics

1. Antigen-expressing single or multi-HLA cell lines



The T-cell epitope profile of the MyT platform is similar to that of human cells

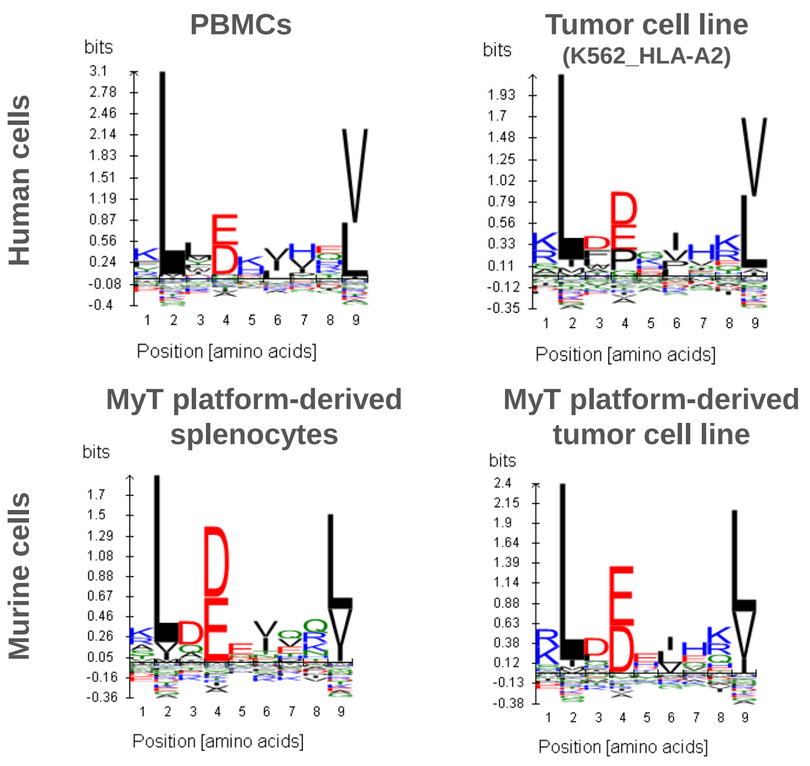


Fig. 1: The HLA-A*02:01-specific motifs of all 9-mer peptides identified from human and murine cells detected by immunopeptidomics are highly comparable between each other and in relation to published motifs. The size of the amino acid letters in the motif are plotted in relation to the general frequency of the respective amino acid in the proteome. The larger the size of the letter, the higher the enrichment of the amino acids as compared to the average frequency.

Immunopeptidomics-identified T-cell epitopes and their relative abundance are similar in the MyT platform and human cells

(Exemplified by the tumor antigen MAGE-A1/HLA-A*02:01)

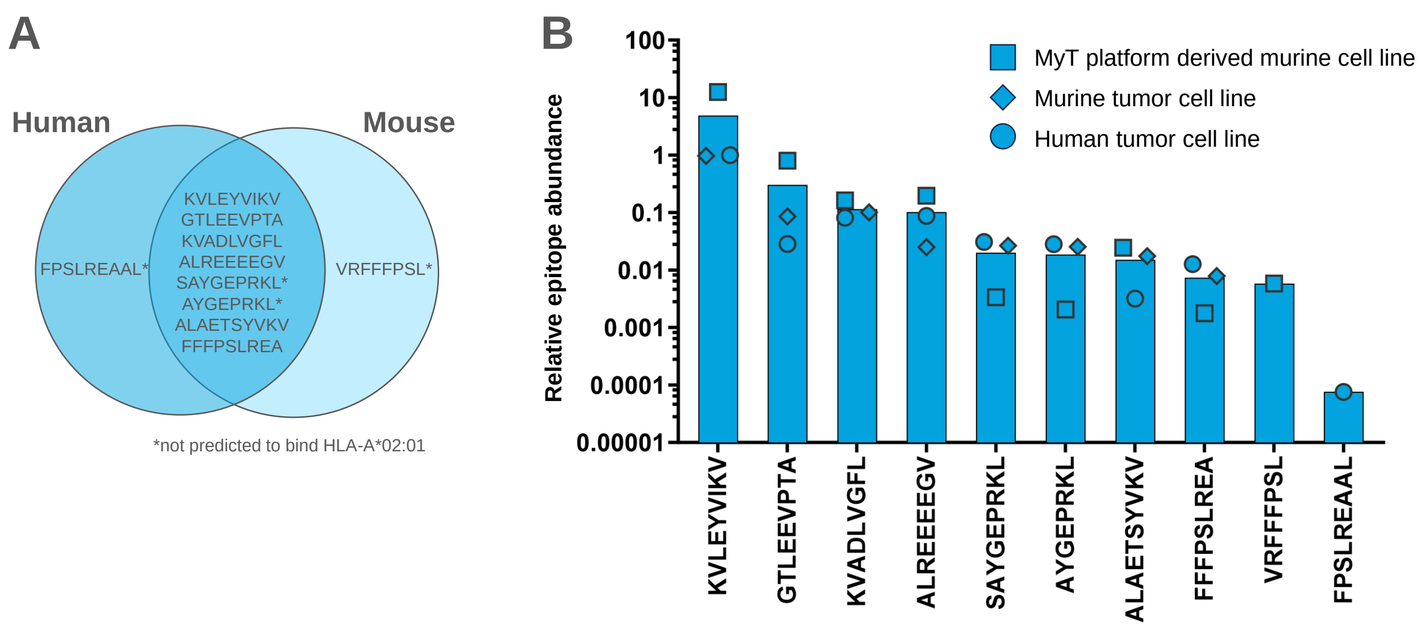


Fig. 2: (A) Immunopeptidomics identified 8 MAGE-A1/HLA-A*02:01-restricted peptides that were found in both **human** K562 cells engineered to express MAGE-A1 and HLA-A*02:01 as well as a MyT platform-derived **murine** cell line showing that epitope processing for MAGE-A1 is highly similar. In addition, 2 low-abundant epitopes were identified that were species-specific. Notably, while most of the peptides could be predicted *in silico*, we found also novel, unpredicted epitopes showing the limitations of using *in silico* approaches for epitope discovery. **(B)** The relative abundance of the peptides was also highly similar between human cells (K562_HLA-A2_MAGE-A1) and murine cells (NIH3T3_HLA-A2_MAGE-A1 and a syngeneic MyT platform-derived fibrosarcoma cell line) suggesting that epitope processing and presentation between humans and mice is highly conserved. Notably, KVL which is targeted in the IMAG1NE trial (NCT05430555), was the highest abundant epitope. Abundance is displayed as molecules/cell relative to KVL on K562_HLA-A2_MAGE-A1.

Epitopes with high abundance on tumor cells have highest immunogenicity in the MyT platform

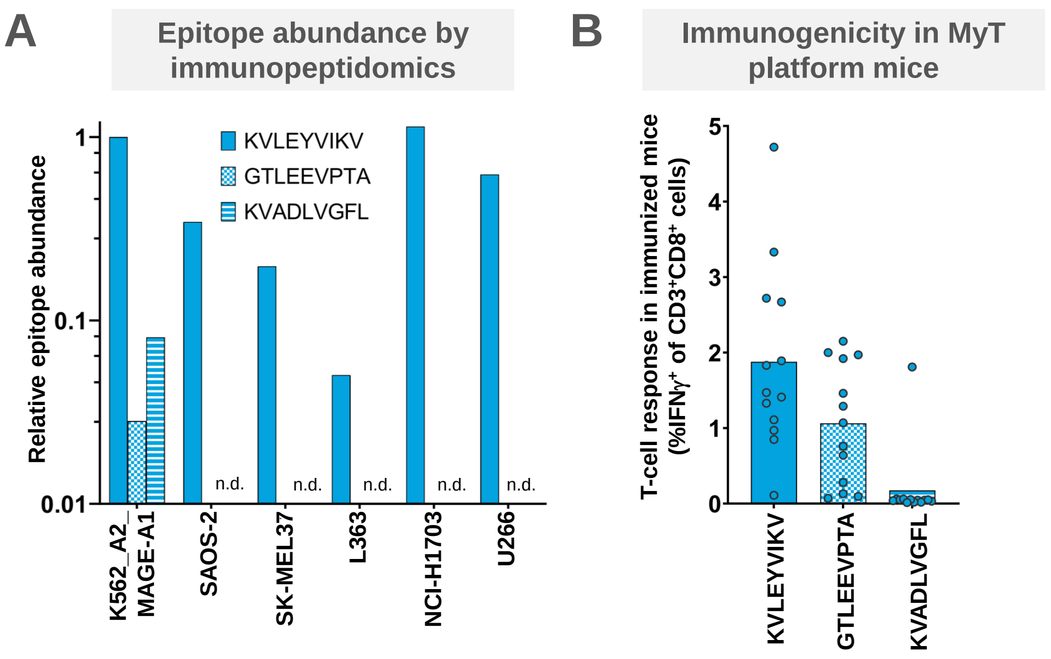


Fig. 3: (A) Immunopeptidomics analysis of multiple human MAGE-A1$^+$/HLA-A2$^+$ tumor cell lines identified only the epitope KVL consistently at high abundance. Only in antigen/HLA overexpressing K562-A2_MAGE-A1 cells, we found additionally the epitopes GTL and KVA, but at substantially lower abundance (displayed as molecules/cell relative to KVL on K562_A2_MAGE-A1). n.d.: not detected **(B)** MyT platform mice (n=13) were immunized with full-length MAGE-A1 antigen and HLA-A*02:01-restricted immune responses were measured by specific restimulation of splenic T-cells and detection of intracellular IFNγ. The T-cell response was dominated by the epitope KVL, while GTL and KVA induced lower and less frequent responses. This shows that the MyT platform selects the most abundant epitopes. Still, differences in abundance and immune response to GTL and KVA also suggest that immunogenicity cannot alone be predicted by surface abundance and that the MyT platform confers additional advantages in epitope discovery and selection.

MyT platform selects the epitopes of highest abundance and immunogenicity among different HLAs

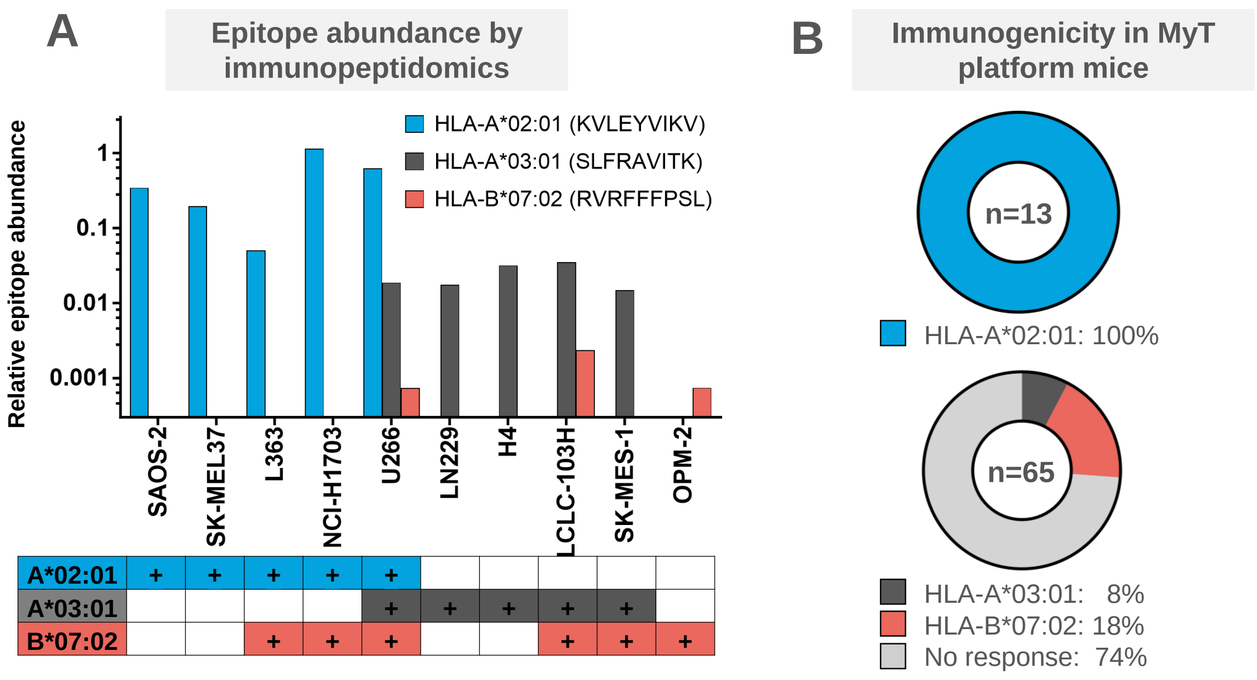


Fig. 4: (A) Immunopeptidomics analysis of multiple MAGE-A1$^+$ tumor cell lines with different HLA allotypes showed that the HLA-A*02:01 epitope KVL was substantially more abundant than two previously described MAGE-A1 TCR-T targets: the epitope SLF restricted to HLA-A*03:01, and RVR restricted to HLA-B*07:02. Abundance is displayed as molecules/cell relative to KVL on K562_A2_MAGE-A1 cells. **(B)** Full-length MAGE-A1 immunization induced strong and frequent HLA-A*02:01-restricted immune responses in MyT platform mice after 2 boosts (top), while responses to HLA-A*03:01 and -B*07:02 were less frequent even after 5 boosts (bottom). These data confirm that epitope immunogenicity in the MyT platform largely reflects epitope abundance supporting its use to select the most suitable target epitopes. Further, both MyT platform and immunopeptidomics studies question the suitability of the SLF and RVR epitopes for TCR-T therapy.

Conclusions

- **T-knife's MyT platform is a powerful tool for the discovery and selection of dominant T-cell epitopes**
- **The T-cell epitope profile of the MyT platform reflects that of human cancer cells**
- **While immunopeptidomics-based epitope discovery determines epitope abundance, immunization with the MyT platform provides additional information on whether an epitope is also immunogenic and can be targeted by T-cells**
- **Targeting the most immunogenic epitopes of a tumor antigen with TCR-T may impact the likelihood of clinical success**
- **In addition to discovery of cancer epitopes, the MyT platform is also suitable for epitope discovery for autoimmune and infectious diseases**

References

Li LP. *et al*. Nat Med 2010;16:1029-1034
Immunopeptidomics data generated by Alithea Bio

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