Exploiting peptide presentation for the development of novel immunotherapies

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Background

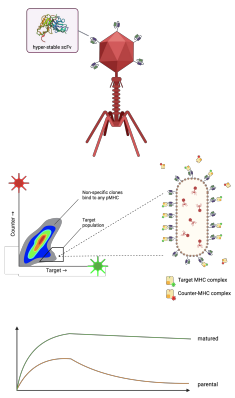
- Most immunotherapies target cell surface proteins preferentially expressed by tumors and not healthy tissue.
- Many high profile targets, such as common oncogenic driver mutations, are found intracellularly and inaccessible to conventional immunotherapeutic approaches.
- However, peptides derived from intracellular proteins that are presented on the cell surface as peptide-Human Leukocyte Antigen (HLA) complexes may be targeted.

- Myrio's platform, **Retained Display (ReD)™**, enables efficient isolation and affinity maturation of specific peptide-HLA complex binders (single chain variable fragments, scFvs), that are readily converted to different therapeutic formats, including CAR and bispecific antibodies.

Peptide classes successfully targeted using ReD™

Neoantigens	Developmental/ cancer testis antigens	Virus-derived	Secreted tumor-associated factors	'Dark antigens'
Driver mutations, E.g. mutant KRAS Gene fusions, E.g. EWS-WT1	E.g. PRAME, PHOX2B	E.g. human endogenous retrovirus (HERV-E), human papilloma virus (HPV16 E7)	E.g. Prostate Specific Antigen (PSA)	E.g. novel epitopes generated by alternative splicing

ReD™ platform[1] for peptide-HLA binder discovery and maturation

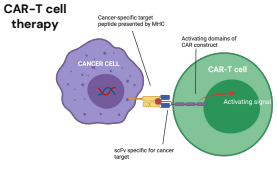


Lambdoid phage display
- Panning of large (>300 billion), fully human scFv library against target peptide-HLA
- High avidity interaction
- Panned phage are used to infect bacteria (one phage per bacterium) for further selection by FACS

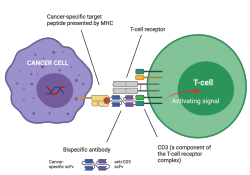
Cell display
- scFv displayed on bacterial cell wall
- >10K clones/second analysed and sorted for target-selective binding
- Counter peptides guide selectivity
- Avoid predicted off-targets early in development

Maturation
- Midi-scFv libraries based on original clone are re-screened for target binding
- Achieve single-digit nM to pM binding affinity
- Known cross-reactive peptides may be included as counters to fine-tune specificity

scFvs can be converted to different therapeutic formats



Various CAR architectures explored in context of peptide-HLA targeting (e.g. PHOX2B PC-CAR[2], and mutant KRAS CAR shown in Fig 5)



Myrio has explored up to 31 different half-life extended TCE formats

Example 1: Isolation of a binder 'breaking restriction' across HLA alleles

- **Target:** 10-mer peptide derived from human endogenous retrovirus type E (HERV-E) presented by HLA-A*11[3]
- **Target indication:** clear cell renal carcinoma (ccRCC)
- An scFv isolated by ReD™ recognizes the same HERV-E peptide presented by HLA-A*11:01 *and* HLA-A*03:01

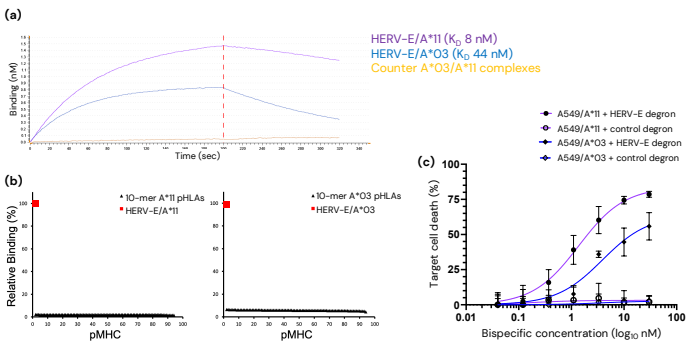


Figure 1: ReD™ scFv library was screened for binders of a 10-mer peptide derived from HERV-E in HLA-A*11. **(a)** Binding kinetics of an isolated scFv to HERV-E/A*11 (purple) and HERV-E/A*03 (blue), assessed using Biolayer Interferometry. **(b)** Target selectivity assessed using arrays of irrelevant 10-mer peptides refolded in A*11 (left) or A*03 (right), relative to target HERV-E/A*11 or A*03, using a bead-based flow cytometric assay. **(c)** scFv was converted to T cell engager format by combining with an anti-human CD3 scFv and evaluated for the ability to mediate T cell killing of luciferase$^+$ A549 cells engineered to endogenously present HERV-E peptide by A*11 (closed circles, purple) or A*03 (closed diamond, blue).

References:
1 Beasley, M. *et al.* (2015), *Biotechnol J*, 10(5): 783-789
2 Yarmarkovich, M. *et al.* (2023), *Nature*, 623(7988): 820-827
3 Takahashi, Y. *et al.* (2008), *J Clin Invest*, 118(3): 1099-1109
4 Bear, A.S. *et al.* (2024), *J Clin Invest*, 134(21):e175790

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Example 2: Isolation of binders targeting a mutant KRAS neoantigen peptide

Target: 10-mer KRAS G12V peptide presented by HLA-A*11:01

Target indications: KRAS G12V$^+$ tumors, e.g. pancreatic, colon, lung cancers

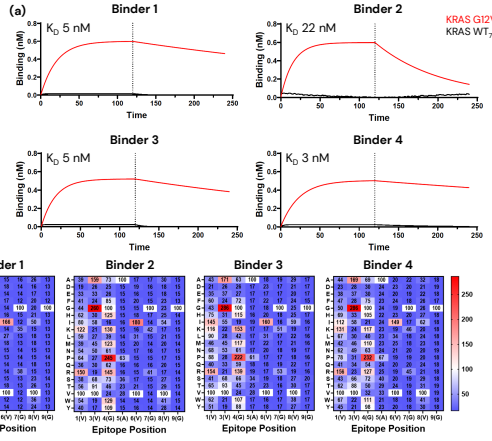
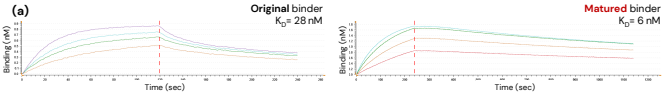


Figure 2: ReD™ scFv library was screened for binders of 10-mer KRAS G12V/A*11, while avoiding wildtype KRAS/A*11. **(a)** Binding kinetics of isolated scFvs to KRAS G12V/A*11 (red) and wildtype KRAS/A*11 (black) complexes assessed using Biolayer Interferometry. **(b)** X-Scan analysis was used to map the 'footprint' of each scFv on the target complex. Each position of the target peptide (excluding anchor residues) was substituted for every amino acid (excluding cysteine) and refolded as pHLA. Binding was assessed using a fluorometric bead-based assay, relative to the target complex.

Affinity matured binders maintain target selectivity

- Target KRAS G12V/A*11 binding affinity improved to single-digit nM following affinity maturation



- Target selectivity maintained following affinity maturation
- No cross-reactivity to predicted off-target peptide/A*11 complexes

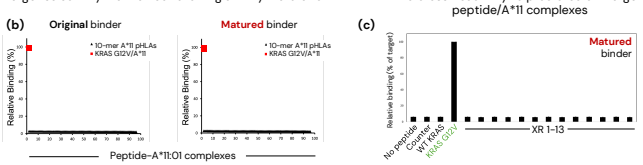


Figure 3: Anti-KRAS G12V/A*11 scFv originally isolated using ReD™ was successfully affinity matured to single-digit nM affinity while maintaining target selectivity. **(a)** Target binding affinity assessed using different concentrations of KRAS G12V/A*11 complex by Biolayer Interferometry. **(b)** Target selectivity assessed using an array of 10-mer peptides refolded in A*11 using a bead-based flow cytometric assay. **(c)** Predicted off-target peptides with homology to KRAS G12V 10-mer peptide were refolded with A*11 and assessed for scFv binding using a bead-based flow cytometric assay.

KRAS G12V/A*11 targeting T cell engager activity *in vitro*

Killing of tumor cells with native and transgenic A*11+ presenting KRAS G12V:

Minimal T cell activation in presence of A*11+ tumor cells lacking KRAS G12V mutation

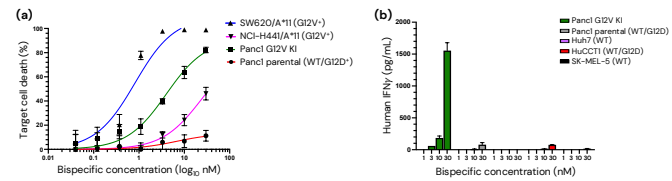


Figure 4: An scFv converted to half-life extended T cell engager (TCE) format was evaluated for activity against KRAS mutant and wild type tumor models. **(a)** Varying degrees of potency against tumor models presenting different levels of KRAS G12V/A*11 target complex[4]. A Panc1 KRAS G12V knockin (KI) model was generated using CRISPR editing of parental Panc1 (A*11+). Killing was assessed by measuring luciferase activity from target cells 48 hours after T cells and antibody were added at 5:1 E:T ratio. **(b)** Using a panel of A*11+ tumor models, IFNγ release was observed only in the presence of KRAS G12V+ cells, but not KRAS wild type of G12D+ cells (measured by ELISA).

KRAS G12V/A*11 CAR-T activity *in vitro*

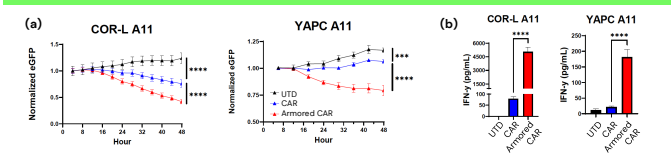


Figure 5: An scFv was converted to CAR and armored CAR formats. Transduced primary T cells were evaluated for activity against KRAS G12V/A*11+ tumor model spheroids over 48 hours. **(a)** Killing of COR-L A*11 and YAPC A*11 spheroids measured using fluorescence microscopy. **(b)** IFNγ release by CAR- and armored CAR-T measured by ELISA.

Conclusions

- Retained Display™ identifies binders of specific peptide-HLA complexes for therapeutic targeting of solid tumors.
- Depending on the desired clinical strategy, scFvs convert easily to different therapeutic formats and can be affinity matured without losing target selectivity.
- scFvs may 'break restriction'; recognising the same target peptide presented by different HLA alleles, which significantly expands eligible patient populations.

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