FairJourneyBio
THE ANTIBODY COMPANY

Generation of anti-pHLA antibodies

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CASE STUDY

TCR-MIMETIC DISCOVERY · PHLA TARGETING

Discriminating mutant from wild-type pHLA at the antibody discovery stage

4
NAÏVE HUMAN LIBRARIES SCREENED IN PARALLEL

53
MUTANT-PHLA-SPECIFIC UNIQUE FVS RECOVERED

82%
MUTANT-SPECIFIC CLONES (BEST LIBRARY)

2.97 nM
LEAD TCE AFFINITY KD ON MUTANT PHLA

BACKGROUND

Targeting intracellular oncoproteins via the HLA class I axis

The therapeutically relevant proteome is dominated by intracellular targets that lie beyond the reach of conventional antibodies. HLA class Imediated antigen processing, however, surfaces fragments of these proteins as peptide–HLA (pHLA) complexes, generating substrates that are accessible to both T-cell receptors (TCRs) and TCR-mimetic (TCRm) antibodies. Mutation-driven oncoproteins are particularly attractive targets within this paradigm: their wild-type (WT) counterparts are presented across healthy tissues, often differing from the mutant peptide by a single residue. A TCRm therapeutic must therefore discriminate between sequences whose only difference may be one amino acid – failure to do so converts on-target activity into systemic on-target/off-tumour toxicity. The therapeutic index for these modalities is set at the discovery stage, not later in development.

STRATEGY

Stringent counter-selection against WT and irrelevant pHLA

Phage display selections were performed against the mutant pHLA target across four FairJourney Bio fully human naïve libraries (each >1×1010 functional diversity; scFv and Fab formats; separated kappa and lambda repertoires). Counter-selection pressure was applied during each round at increasing concentrations of irrelevant and WT pHLA, depleting cross-reactive phage before positive selection on the mutant target (Figure 1). Stringency was titrated empirically: as counter-selection concentration rose, fold-enrichment dropped sharply for libraries skewed toward non-specific binders, while libraries enriched in genuinely mutant-discriminating clones retained signal – providing an early read on which library outputs were worth carrying into screening.

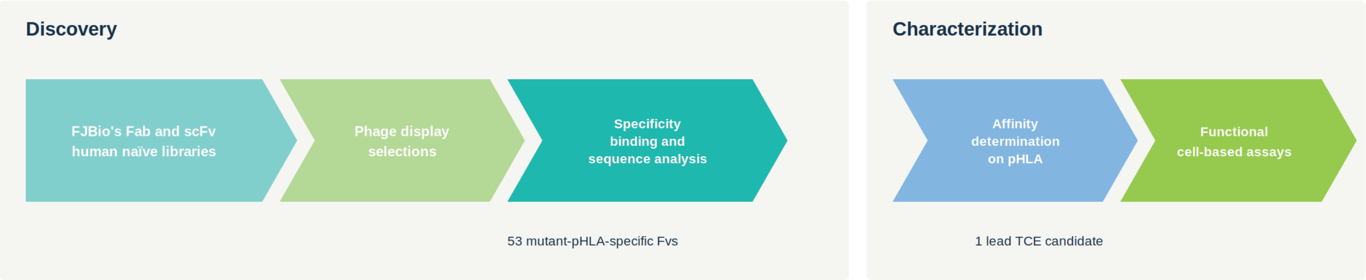


Figure 1. End-to-end workflow. Four naïve human libraries (Fab and scFv) feed phage display selections under graded counter-selection pressure with irrelevant and WT pHLA. Output is triaged by ELISA-based specificity screening, HCDR3 sequence analysis, and FACS confirmation on T2 cells. The lead Fv is reformatted as a T-cell engager (TCE) and fully characterised in-house — affinity by SPR, function in cell-based assays.

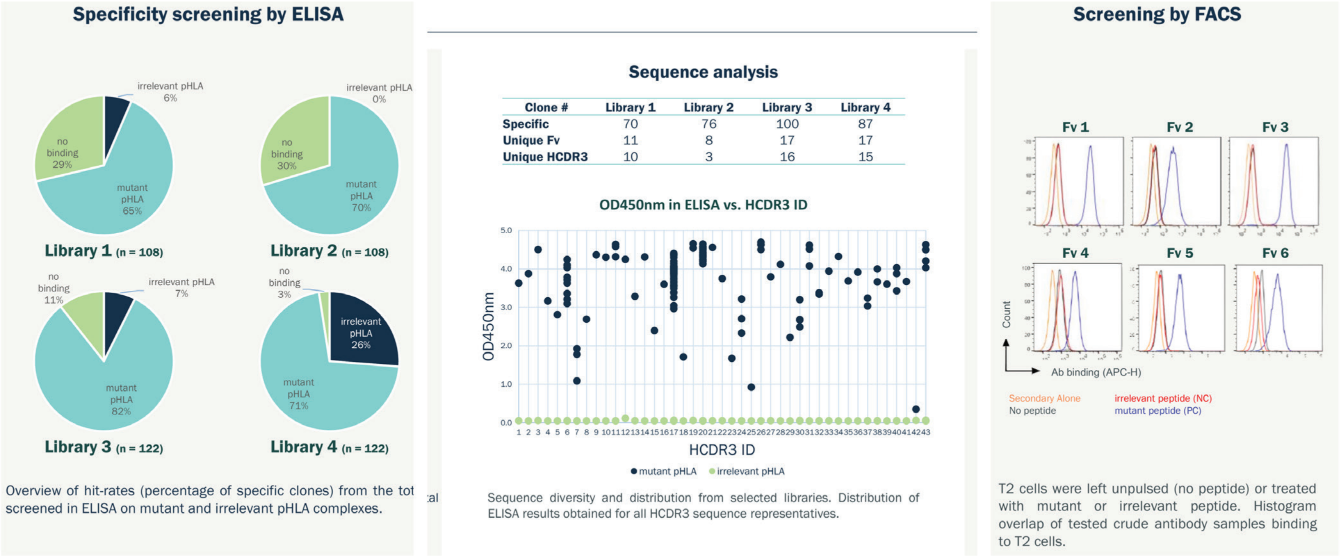
RESULTS

Discovery Output — 53 Mutant-Phla-Specific Fvs Across Four Libraries

All four libraries returned mutant-pHLA-specific clones, but with markedly different specificity yields and uniqueness profiles (Table 1). Library 3 emerged as the cleanest output: 82% of screened clones bound the mutant pHLA in ELISA with negligible irrelevant-pHLA crossreactivity, resolving to 17 unique Fvs and 16 unique HCDR3 sequences. Library 4, run at lower counter-selection stringency, returned 71% mutant-specific but with 26% irrelevant-pHLA binding still present – illustrating directly the cost of insufficient counter-selection pressure. FACS on T2 cells pulsed with mutant peptide, irrelevant peptide, or unpulsed confirmed cellular-level specificity for the lead clones (Figure 2). 53 unique mutant-pHLA-specific Fvs were taken forward.

Library	Clones screened	Mutant pHLA	Irrelevant pHLA	Specific clones	Unique Fv	Unique HCDR3
Library 1	108	65%	6%	70	11	10
Library 2	108	70%	0%	76	8	3
Library 3	122	82%	7%	100	17	16
Library 4	122	71%	26%	87	17	15
Total	**460**	**—**	**—**	**333**	**53**	**44**

Table 1. *Per-library specificity yield from ELISA screening on mutant vs. irrelevant pHLA, with downstream sequence diversity. Library 3 (highlighted) returned the highest specificity yield and the largest pool of unique HCDR3 sequences.*



Overview of hit-rates (percentage of specific clones) from the tot
screened in ELISA on mutant and irrelevant pHLA complexes.

Sequence analysis

Clone #	Library 1	Library 2	Library 3	Library 4
Specific	70	76	100	87
Unique Fv	11	8	17	17
Unique HCDR3	10	3	16	15

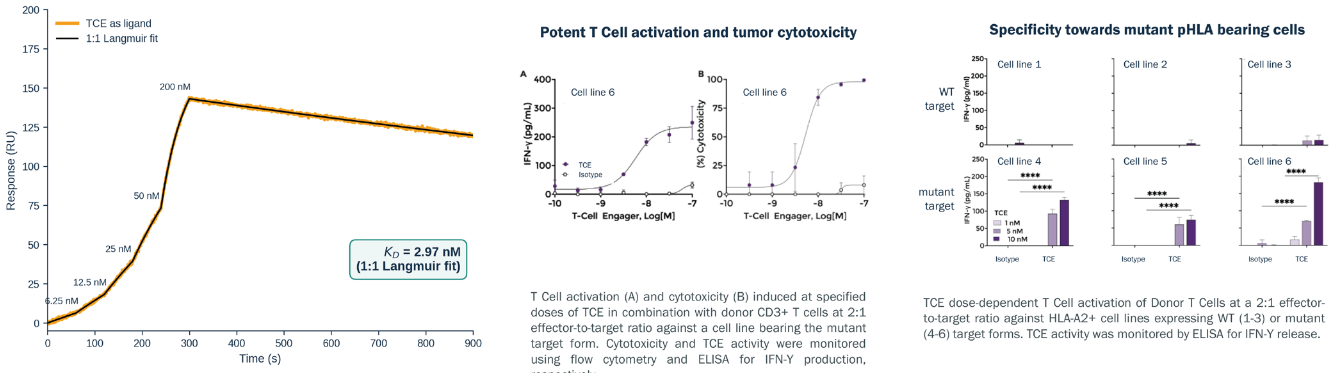
Sequence diversity and distribution from selected libraries. Distribution of ELISA results obtained for all HCDR3 sequence representatives.

T2 cells were left unpulsed (no peptide) or treated with mutant or irrelevant peptide. Histogram overlap of tested crude antibody samples binding to T2 cells.

Table 2. *Hit triage.* ***Left:*** *per-library hit-rates by ELISA on mutant vs. irrelevant pHLA.* ***Centre:*** *HCDR3 diversity and ELISA OD450 nm distribution per unique sequence.* ***Right:*** *FACS on T2 cells pulsed with mutant peptide (PC), irrelevant peptide (NC), or unpulsed — confirming cellular-level specificity.*

Lead TCE — Nanomolar Affinity, Mutant-Restricted T-Cell Killing

The lead Fv was reformatted as a T-cell engager (anti-pHLA × anti-CD3 architecture) and characterised end-to-end in-house. Affinity to the mutant pHLA was measured by SPR single-cycle kinetics across a 6–200 nM range, returning KD = 2.97 nM under a 1:1 Langmuir fit. The TCE drove dose-dependent IFN-γ release and target-cell killing against HLA-A2+ cells presenting the mutant target at a 2:1 effector-to-target ratio. Specificity was tested across a six-cell-line panel — three lines presenting the WT target, three presenting the mutant target. Cytotoxicity and IFN-γ release were strictly confined to the mutant-bearing lines: WT-presenting lines remained at baseline IFN-γ across the full TCE dose range tested (1–10 nM), confirming that the mutant-versus-WT discrimination established at the Fv selection stage was preserved in the final TCE format (Figure 3).



T Cell activation (A) and cytotoxicity (B) induced at specified doses of TCE in combination with donor CD3+ T cells at 2:1 effector-to-target ratio against a cell line bearing the mutant target form. Cytotoxicity and TCE activity were monitored using flow cytometry and ELISA for IFN-Y production,

TCE dose-dependent T Cell activation of Donor T Cells at a 2:1 effector-to-target ratio against HLA-A2+ cell lines expressing WT (1-3) or mutant (4-6) target forms. TCE activity was monitored by ELISA for IFN-Y release.

Figure 3. TCE characterisation. Left: Single-cycle SPR sensorgram of TCE binding immobilized mutant pHLA, with the 1:1 Langmuir fit overlaid. Fivestep injection ladder spanning the 6.25–200 nM range; KD = 2.97 nM. Centre: dose-dependent IFN-γ release (A) and cytotoxicity (B) against HLAA2+ mutant-target-bearing cells (cell line 6) at 2:1 effector-to-target ratio. Right: TCE activity confined to mutant-target-bearing lines (cell lines 4–6); no specific activation on WT-target-bearing lines (cell lines 1–3) at TCE doses up to 10 nM. Significance: **** $p < 0.0001$.

DISCUSSION

Implications for safe TCRm therapeutic development

For mutant-pHLA targets, the therapeutic window is set at the moment of selection: any cross-reactive clone admitted into the discovery output will, in a TCE format, drive cytotoxicity in healthy tissue. Counter-selection at the phage-library stage is the cleanest place to impose that filter, because off-target binders are removed before commitment to a candidate is made. Two further considerations follow. First, peptide-level cross-reactivity to sequence-related but non-target pHLAs cannot be excluded by WT counter-selection alone – the short length of MHC-I peptides means that residues outside the mutation hotspot may permit cross-recognition of unrelated self-peptides. Downstream off-target panels (broader healthy-issue pHLA repertoires; in-silico cross-reactivity prediction) remain essential for clinical stage candidates. Second, format flexibility matters: the same Fv must retain its specificity profile across IgG, bispecific, and TCE architectures. The end-to-end workflow demonstrated here – naïve-library discovery, counter-selection-driven specificity, and in-house TCE reformatting and characterisation – closes that gap by maintaining a single experimental thread from library to functional candidate.

BOTTOM LINE

Discovery-stage counter-selection against WT and irrelevant pHLA, executed across four fully human naïve libraries and confirmed by orthogonal screening, delivers TCRm Fvs that retain mutant-versus-WT discrimination through TCE eformatting and into functional cell-killing assays – the prerequisite specificity profile for translating a TCRm modality into the clinic.

Discuss your TCR-mimetic discovery programme with our team:

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