Anti-Idiotypic Antibody Discovery Case Study

Identifying Specific mAb Binders to a CAR, Enabling Drug Quantification in Patient Serum Sample

ANTI-ID ANTIBODY DISCOVERY FOR CELL-BASED BIOLOGICS

Case study: identifying specific mAb binders to a CAR, enabling drug quantification in patient serum sample

Background

- Partner required a diverse panel of anti-idiotypic mAbs against the antigenic determinant of 'CAR-T X' to support functional measurements in an upcoming clinical trial
- CAR-T X is a T cell-based therapeutic which features a scFv as the antigenic determinant for tumor cell engagement through Surface Target Y (Figure 1)

Requirements for Assays Reagent Development

- A predictable timeline for antibody development pathway
- Specificity against Target scFv (extracellular domain) of the CAR
- Maintenance of binding activity in a complex mixture of PBMCs (human serum)
- On-cell binding to CAR-T X
- A range of affinities

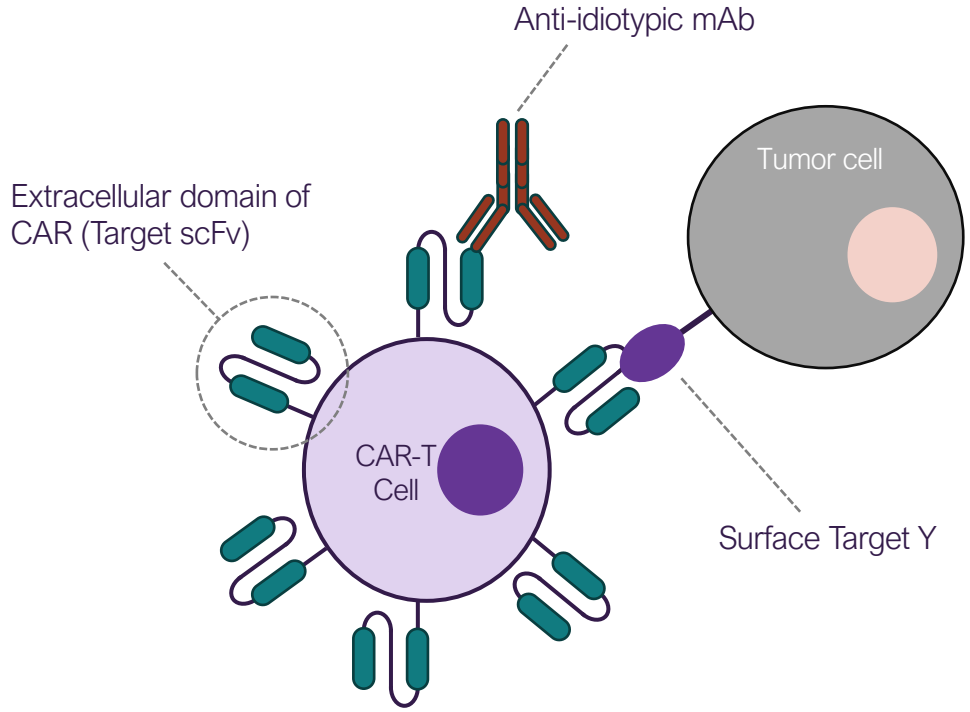
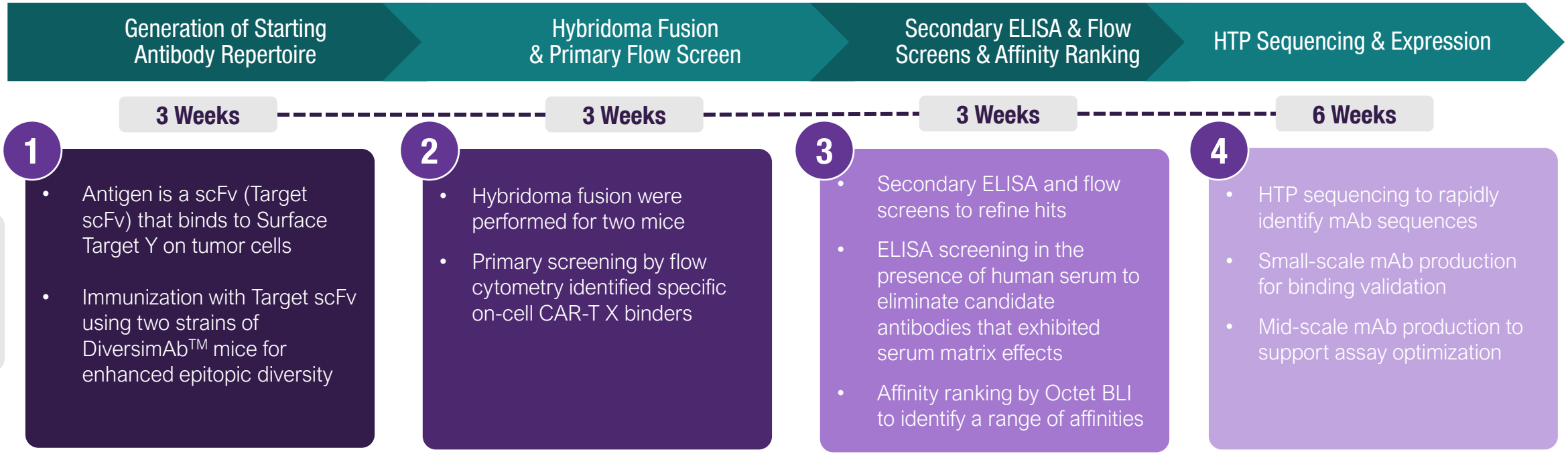
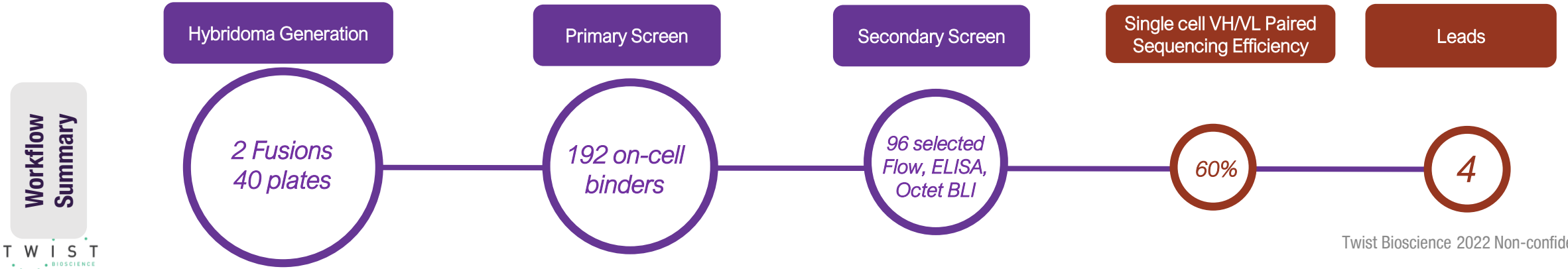


Figure 1. Schematic presentation of anti-idiotypic mAb binding to the extracellular domain (scFv) of CAR-T X, a cell-based therapeutic that functions via binding to Surface Target Y on tumor cell surface

CASE STUDY: HYBRIDOMA APPROACH FOR ANTI-ID AGAINST CAR-T X

A discovery workflow combining broadened antibody diversity and stringent screening to identify ideal mAbs





CASE STUDY: HYBRIDOMA APPROACH FOR ANTI-ID AGAINST CAR-T X

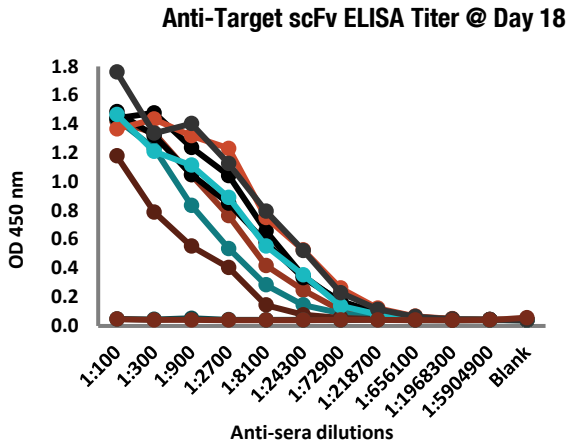
Antigen-specific titers were confirmed by both ELISA and flow cytometry to enable selection of mice for fusion

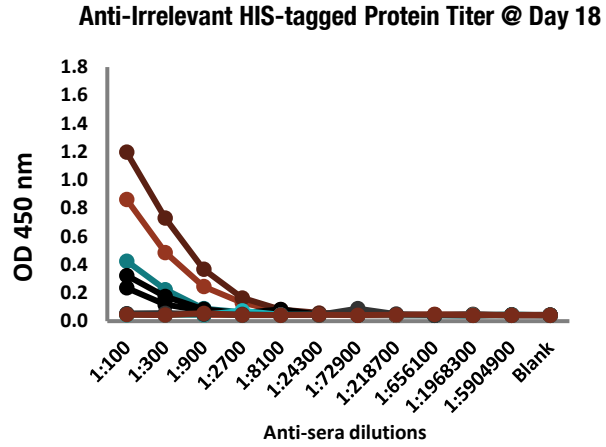
3 Weeks

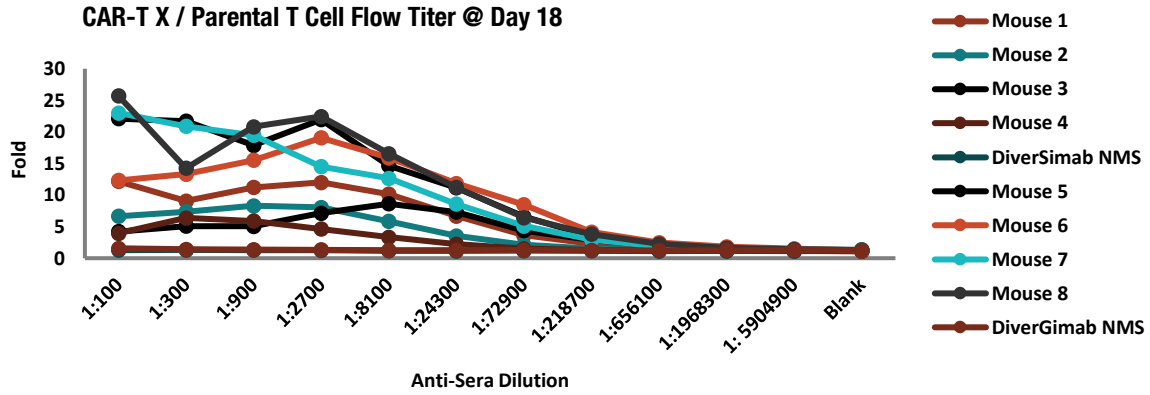
Generation of Starting Antibody Repertoire	Hybridoma Fusion & Primary Flow Screen	Secondary ELISA & Flow Screens & Affinity Ranking	HTP Sequencing & Expression

Immunization and Titer Analysis

- Proprietary accelerated immunization (3 weeks) of DiversimAb™ mice using a His-tagged Target scFv
- Expanded starting repertoire diversity due to multiple strain backgrounds from the DiversimAb family (DiversimAb and DivergimAb)
- Titer testing via ELISA and flow cytometry
- Tissue harvest from two mice (#3 and #6) for hybridoma fusion







CASE STUDY: HYBRIDOMA APPROACH FOR ANTI-ID AGAINST CAR-T X

Flow and ELISA screening cascade revealed specific on-cell and recombinant protein binders to Target scFv with limited serum matrix effects

3 Weeks | 3 Weeks

Generation of Starting Antibody Repertoire → Hybridoma Fusion & Primary Flow Screen → Secondary ELISA & Flow Screens & Affinity Ranking → HTP Sequencing & Expression

Hybridoma Fusion and Primary Flow Screen

- Two hybridoma fusion were performed
- A total of 40, 96-well plates were screened by HTP flow cytometry to identify 192 clones with preliminary on-cell binding for secondary screen

Secondary ELISA Screen Results

- ELISA screen against Target scFv in the presence of 20% human serum protein to test for serum matrix interference (Table 1)
- ELISA counterscreen against irrelevant his-tagged protein in secondary screen to eliminate non-specific binders (Table 1)

Clone	His-Tagged Target scFv (+)	His-Tagged Target scFv in 20% Human Serum (+)	His-Tagged Off-Target scFv (-)	Polyclonal HulgG (-)	On-Cell Binding by Flow (CAR-T X to Parental Cell Line)
1	1.677	1.814	1.522	0.042	9.5
2	1.326	1.155	0.049	0.043	11.3
3	1.322	1.117	0.049	0.042	13.2
4	1.221	0.885	0.941	0.048	11.4
5	1.211	0.99	0.273	0.042	10.3
6	1.149	1.103	0.051	0.044	11.7
7	1.143	0.755	0.049	0.043	9.9
8	1.091	0.963	0.065	0.075	8.7
9	1.059	0.862	0.064	0.043	11.3
10	1.055	0.711	0.068	0.042	2.6
11	1.054	0.807	0.054	0.043	12.3
12	1.033	0.757	0.067	0.042	16.0
13	1.014	0.529	1.54	0.043	15.6
14	1.01	1.184	0.073	0.043	9.6
15	0.974	0.854	0.064	0.046	15.6
16	0.963	0.353	0.059	0.048	10.3
17	0.963	0.42	0.053	0.044	10.8
18	0.93	0.568	0.049	0.054	14.5
19	0.926	0.655	0.066	0.042	15.7
20	0.925	0.618	0.333	0.041	10.2

Table 1. Top 20 clones from secondary screen ranked by target scFv ELISA

CASE STUDY: HYBRIDOMA APPROACH FOR ANTI-ID AGAINST CAR-T X

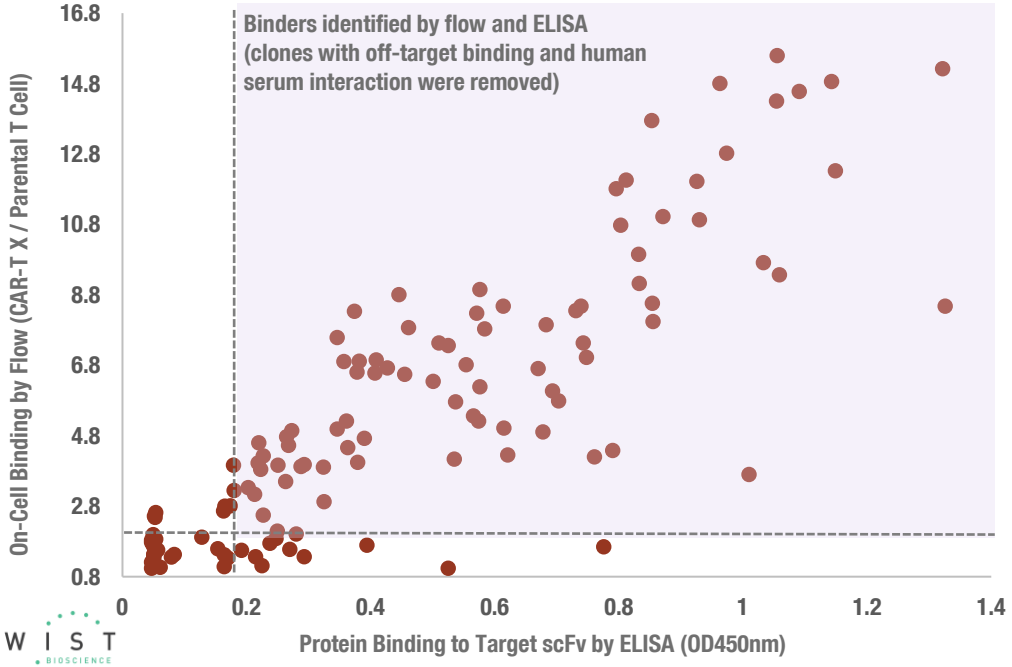
Additional secondary flow screening confirmed cell-binding prior to off-rate ranking via Octet BLI

3 Weeks

Generation of Starting Antibody Repertoire → Hybridoma Fusion & Primary Flow Screen → **Secondary ELISA & Flow Screens & Affinity Ranking** → HTP Sequencing & Expression

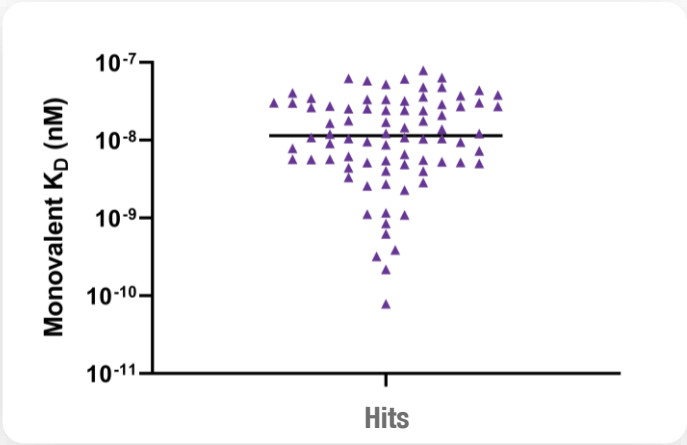
Secondary Flow Screen for On-Cell Binding Confirmation

- Secondary screening revealed a positive correlation between recombinant protein and on-cell binding
- Top 96 clones with specific recombinant protein and on-cell binding were selected for affinity ranking

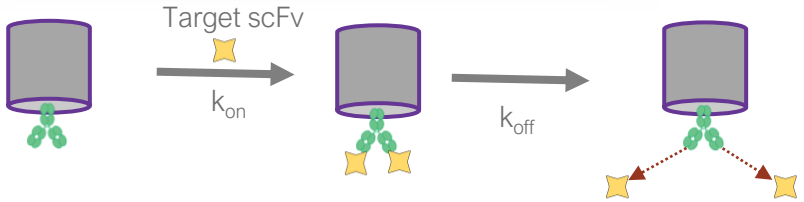


Affinity Ranking by Octet BLI

- Single point kinetics revealed a diverse range of affinities from subnanomolar to double-digit nanonmolar



Affinity ranking by off-rate



CASE STUDY: HYBRIDOMA APPROACH FOR ANTI-ID AGAINST CAR-T X

Delivery of diverse antibodies and purified IgG supported Partner's assay needs

6 Weeks

Generation of Starting Antibody Repertoire → Hybridoma Fusion & Primary Flow Screen → Secondary ELISA & Flow Screens & Affinity Ranking → HTP Sequencing & Expression

Sequencing Results:

- 80 parental hybridomas were sequenced via HTP sequencing while Partner simultaneously validated the clones
- 47 paired VH/VL sequences (42 unique sequences) were recovered in 2 weeks

Expression & Project Delivery:

- 4 clones were selected for small-scale expression (~ 200 μg) and Partner validation (in 4 weeks)
- Mid-scale production (2mg) for 2 final antibodies to provide longer-term supply for assay development (additional 4 weeks)

Conclusions:

- Successful discovery of a highly diverse panel of over 80 specific CAR-T X on-cell binders with nanomolar to subnanomolar affinities
- Validated antibodies with confirmed on-cell binding activity and lack of matrix effects when screened in the presence of human serum
- Rapid sequence recovery with outstanding diversity (89% unique sequences) was obtained from parental hybridomas using HTP sequencing
- Secured supply of purified reagent antibodies in 15 weeks from the start of immunization

Parental clones identified for HTP sequencing

80 clones with confirmed on-cell binding and known Octet BLI affinity ranking profile

↓

47 VH/VL paired sequences (60% recovery)

↓

4 small-scale expressions
~ 200 ug for Partner validation

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Mid-scale production of 2 mg for 2 final antibodies to support Partner's assay development