

Case Study 1

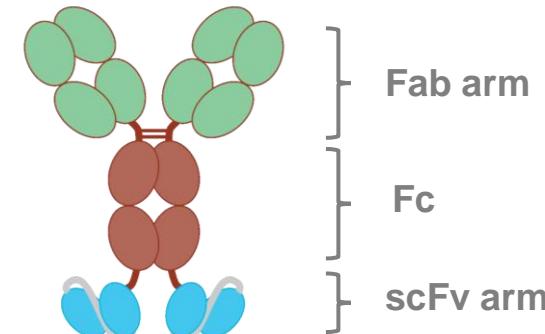
Hybridoma-based Anti-idiotypic mAb Discovery
against a Bispecific Therapeutics for PK/ADA
Assays Development

CASE STUDY ON ANTI-IDIOTYPIC ANTIBODY DISCOVERY

A campaign for the discovery for panels of anti-idiotypic mAb's for a bispecific molecule

Target and Applications:

- Therapeutic bispecific target (IgG-scFv) with two antigen-binding arms (Figure 1)
- Anti-Fab (Cohort 1) and anti-scFv (Cohort 2) antibodies required for PK/ADA assay development
- Unique antibody sequences and antibodies delivered to partner



Antibody characteristics desired

High specificity to the respective antigen-binding arm

Antibodies that works as pairs for bridging assay development (Figure 2)

A range of affinities for assay sensitivity (from sub-nanomolar to double-digit nanomolar affinities)

Optional: Identify neutralizers and non-neutralizers

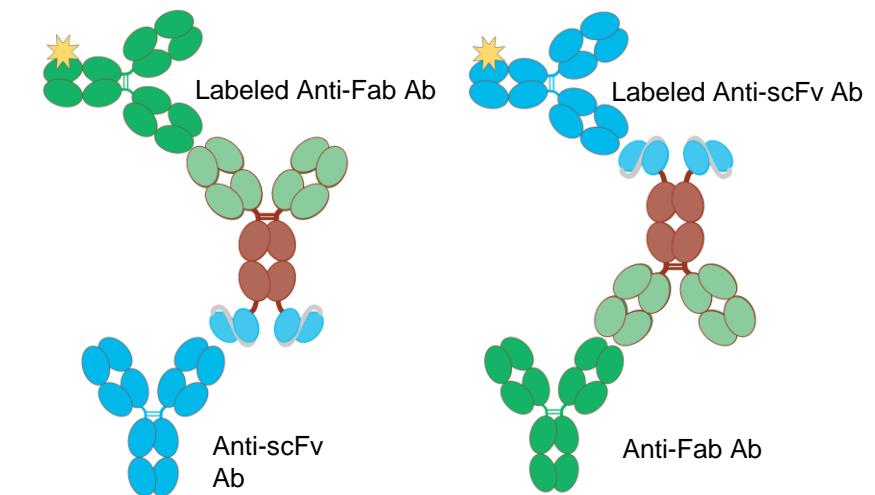


Figure 2. Illustration of the two formats of bridging assay with anti-Fab and anti-scFv as detection or capture antibodies

ANTI-IDIOTYPIC ANTIBODY DISCOVERY – IMMUNIZATION TO SEQUENCES

Workflow for two antigen-binding arms using two cohorts of mice: Cohort 1 (anti-Fab); Cohort 2 (anti-scFv)

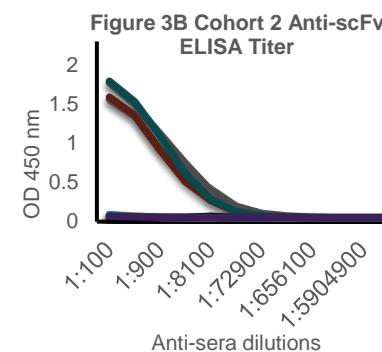
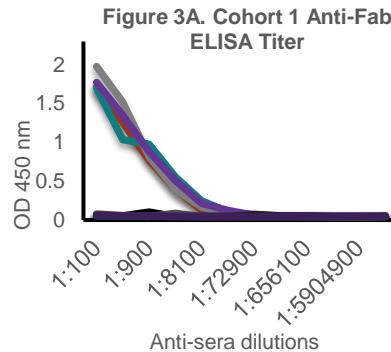


GENERATION OF ANTIBODIES FOR SPECIFICITY SCREENING

Robust immune from DiversimAb mouse allows for generation of hybridoma for screening

Immunization & Hybridoma Generation

Titer check of immunization

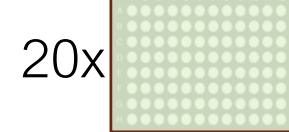


Cohort 1: Fab immunized for anti-Fab antibodies (Figure 3A)
 Cohort 2: scFv immunized for anti-scFv antibodies (Figure 3B)

- Rapid immunization in 18 days
- Good immune response for both cohorts
- 2 mice from each cohort were selected for fusion based on ELISA titer responses
- Lymph tissues were harvested and fused with myeloid cells by electrofusion

ELISA Specificity Screens

Primary screen

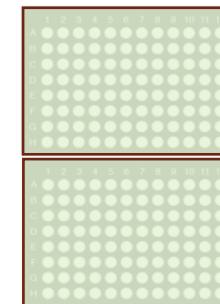


1760 wells
for each cohort

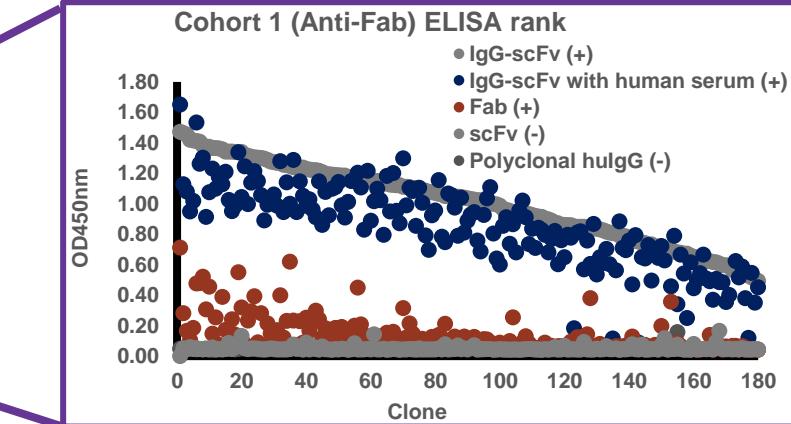
Cohort 1 & Cohort 2

Bispecific target (+)
Polyclonal human IgG (-)

Secondary screen



182 clones
for each cohort



	Cohort 1 Anti-Fab	Cohort 2 Anti-scFv
Bispecific target	(+)	(+)
Bispecific target with 20% human serum	(+)	(+)
Fab	(+)	(-)
scFv	(-)	(+)
Polyclonal hulgG	(-)	(-)

ELISA SECONDARY SPECIFICITY SCREEN RESULTS

Interrogation of the 182 positive hits by a custom screen cascade allows for the selection of top 96 hits

Cohort 1 (Fab immunized)

Clone	Bispecific target (IgG-scFv) (+)	Bispecific target with 20% human serum (+)	Fab (+)	scFv (-)	Polyclonal hulgG (-)
1-1	1.474	1.653	0.713	0.048	0.049
1-2	1.466	1.129	0.282	0.052	0.051
1-3	1.414	1.536	0.478	0.053	0.044
1-4	1.408	1.264	0.479	0.046	0.046
1-5	1.384	1.304	0.523	0.044	0.044
1-6	1.378	0.915	0.308	0.052	0.045
1-7	1.375	1.077	0.456	0.046	0.043
1-8	1.366	1.101	0.256	0.045	0.043
1-9	1.359	1.129	0.389	0.045	0.044
1-10	1.343	0.953	0.243	0.047	0.046
1-11	1.341	1.339	0.551	0.076	0.047
1-12	1.341	1.046	0.320	0.134	0.076
1-13	1.317	0.999	0.235	0.046	0.051
1-14	1.311	1.139	0.296	0.051	0.05
1-15	1.305	1.215	0.393	0.052	0.049

Table 1A. 15 representative clones from secondary screen cascade ranked by bispecific target (+) readout

Cohort 2 (scFv immunized)

Clone	Bispecific target (IgG-scFv) (+)	Bispecific target with 20% human serum (+)	Fab (-)	scFv (+)	Polyclonal hulgG (-)
2-1	1.411	0.265	0.043	1.346	0.049
2-2	1.408	0.802	0.05	1.487	0.051
2-3	1.382	0.543	0.042	1.409	0.05
2-4	1.346	0.273	0.049	1.749	0.183
2-5	1.325	0.161	0.043	1.183	0.052
2-6	1.316	0.462	0.042	1.292	0.044
2-7	1.286	0.659	0.042	1.207	0.047
2-8	1.285	0.501	0.042	1.22	0.046
2-9	1.273	0.423	0.055	1.337	0.051
2-10	1.265	0.375	0.042	1.221	0.045
2-11	1.244	0.095	0.048	1.099	0.053
2-12	1.224	0.871	0.045	0.99	0.053
2-13	1.223	0.577	0.042	1.185	0.049
2-14	1.211	0.326	0.061	1.25	0.056
2-15	1.206	1.026	0.049	1.291	0.06

Table 1B. 15 representative clones from secondary screen cascade ranked by bispecific target (+) readout

- Ranking of 182 clones screen by readout of anti-bispecific target ELISA in secondary screen
- Screen cascade allows for the selection of binders with stringent specificity requirements
- 96 hits were selected for affinity ranking for each cohort

ELISA SECONDARY SPECIFICITY SCREEN RESULTS

Screening for the retainment of binding in the presence of human serum is critical

Several antibodies appeared specific to the bispecific and the ScFv then showed reduced binding capacity in the presence of human serum.

Screening in the presence of human serum early enables downselection of mAbs that are not suitable for clinical assay use

Cohort 2 (scFv immunized)

Clone	Bispecific target (IgG-scFv) (+)	Bispecific target with 20% human serum (+)	Fab (-)	scFv (+)	Polyclonal hulgG (-)
2-1	1.411	0.265	0.043	1.346	0.049
2-2	1.408	0.802	0.05	1.487	0.051
2-3	1.382	0.543	0.042	1.409	0.05
2-4	1.346	0.273	0.049	1.749	0.183
2-5	1.325	0.161	0.043	1.183	0.052
2-6	1.316	0.462	0.042	1.292	0.044
2-7	1.286	0.659	0.042	1.207	0.047
2-8	1.285	0.501	0.042	1.22	0.046
2-9	1.273	0.423	0.055	1.337	0.051
2-10	1.265	0.375	0.042	1.221	0.045
2-11	1.244	0.095	0.048	1.099	0.053
2-12	1.224	0.871	0.045	0.99	0.053
2-13	1.223	0.577	0.042	1.185	0.049
2-14	1.211	0.326	0.061	1.25	0.056
2-15	1.206	1.026	0.049	1.291	0.06

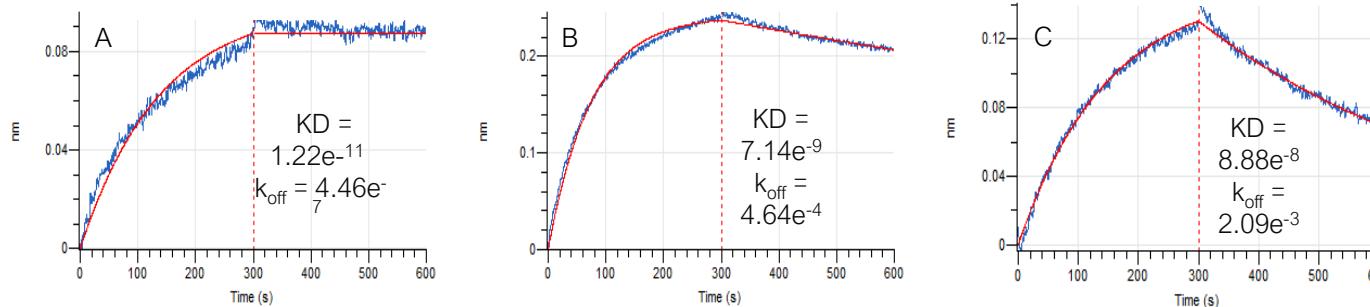


Quality of anti-ID for Cohort 2 (anti-scFv) is significantly improved by screening out low readouts in the presence of human serum

OCTET BLI AFFINITY MEASUREMENT RESULTS

Affinity ranked by off-rate by direct measurement on crude supernatant of the hybridoma suspensions

Figure 5A Cohort 1 (anti-Fab) Binding Kinetics Representative Examples



Cohort 1 k_{off} Ranking

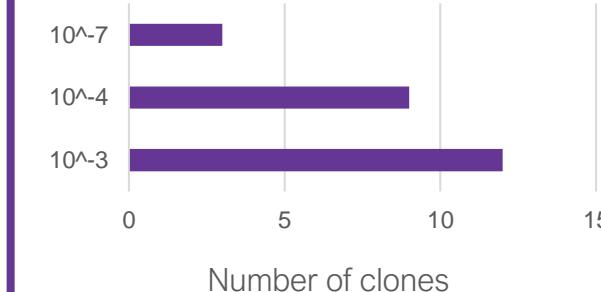
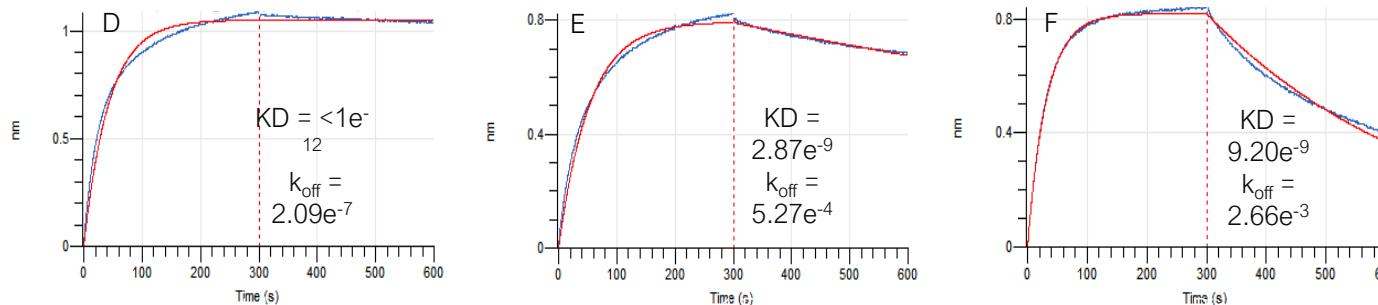
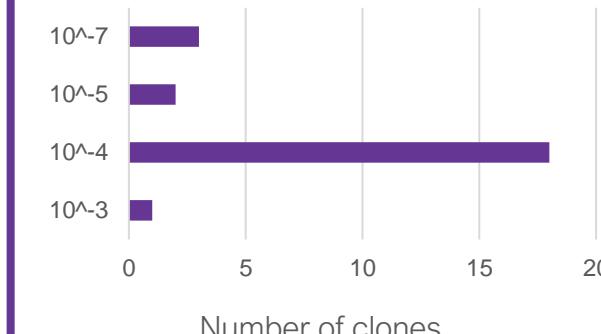


Figure 5B. Cohort 2 (anti-scFv) Binding Kinetics Representative Examples



Cohort 2 k_{off} Ranking

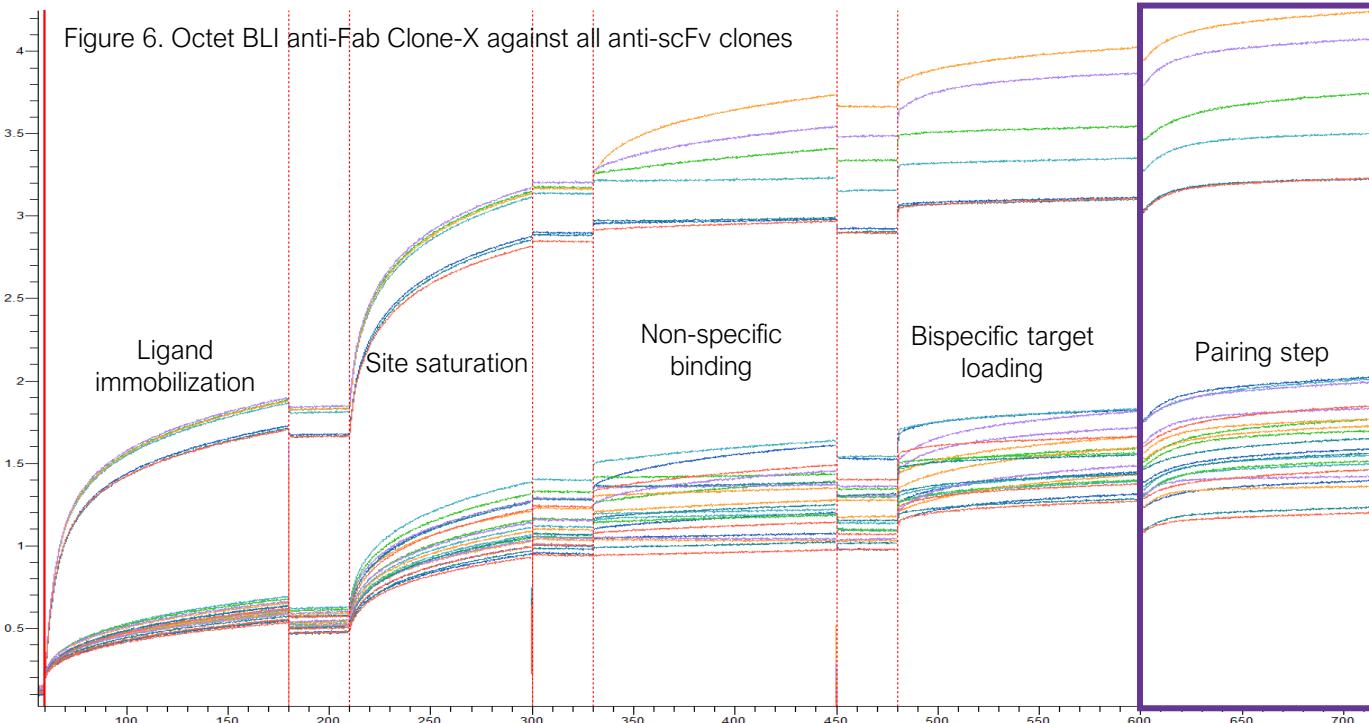
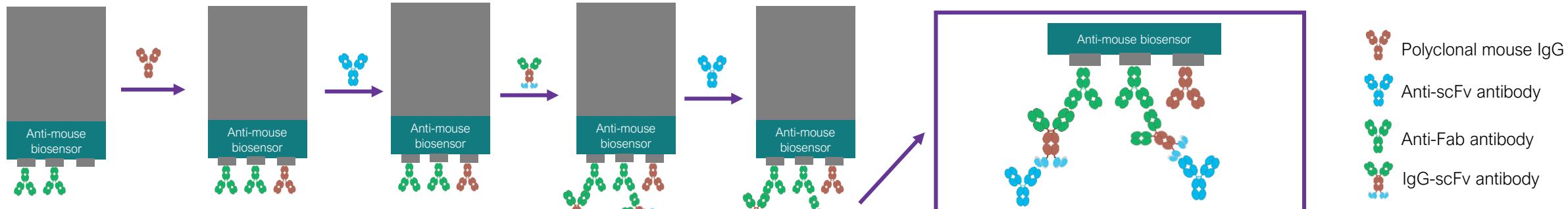


Conclusion

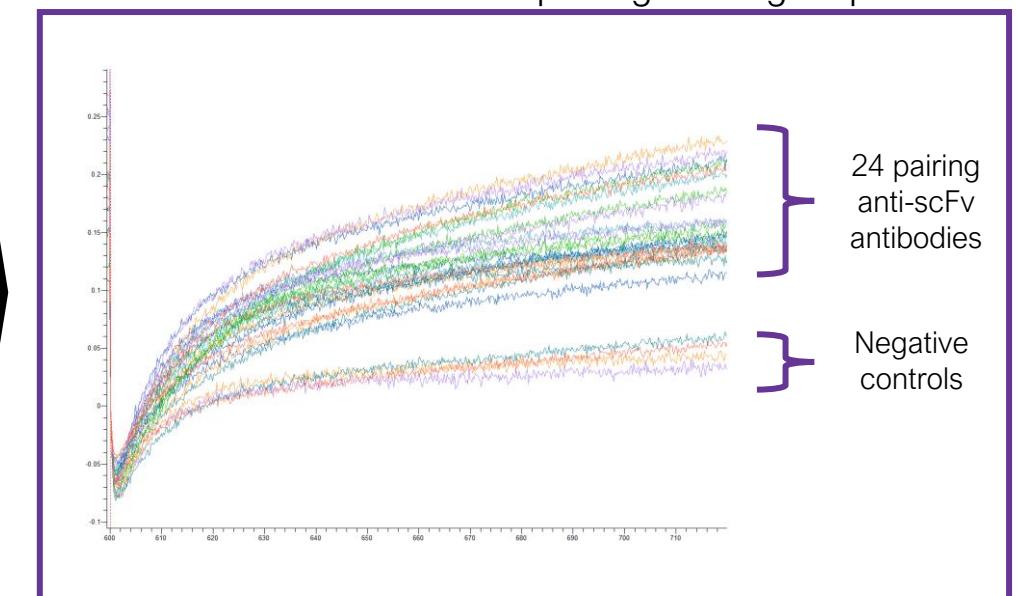
Two panels of clones with good ranges of affinity were ranked by off-rate
24 antibodies from each were selected to move on for pairing analysis

IDENTIFYING ANTIBODY PAIRS THAT BIND TO THE IgG-scFv

A loading sequence is designed to detect anti-Fab and anti-scFv that will bind to the antibodies



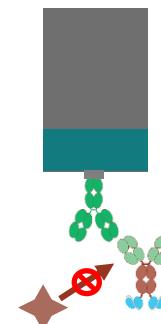
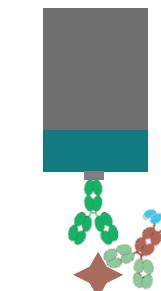
Normalized view of the pairing binding step



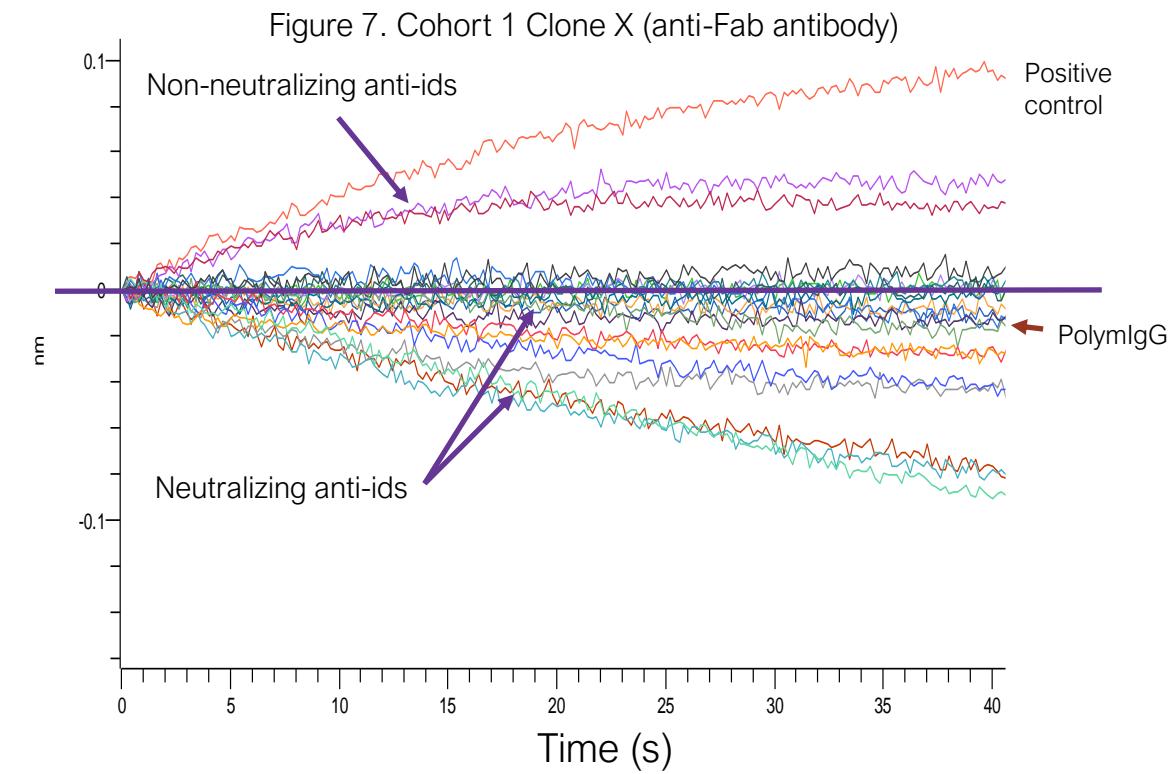
IDENTIFICATION OF NEUTRALIZERS/NON-NEUTRALIZERS

Successful identification of non-neutralizer for anti-Fab antibodies

2 non-neutralizers were identified in Cohort 1 (anti-Fab)



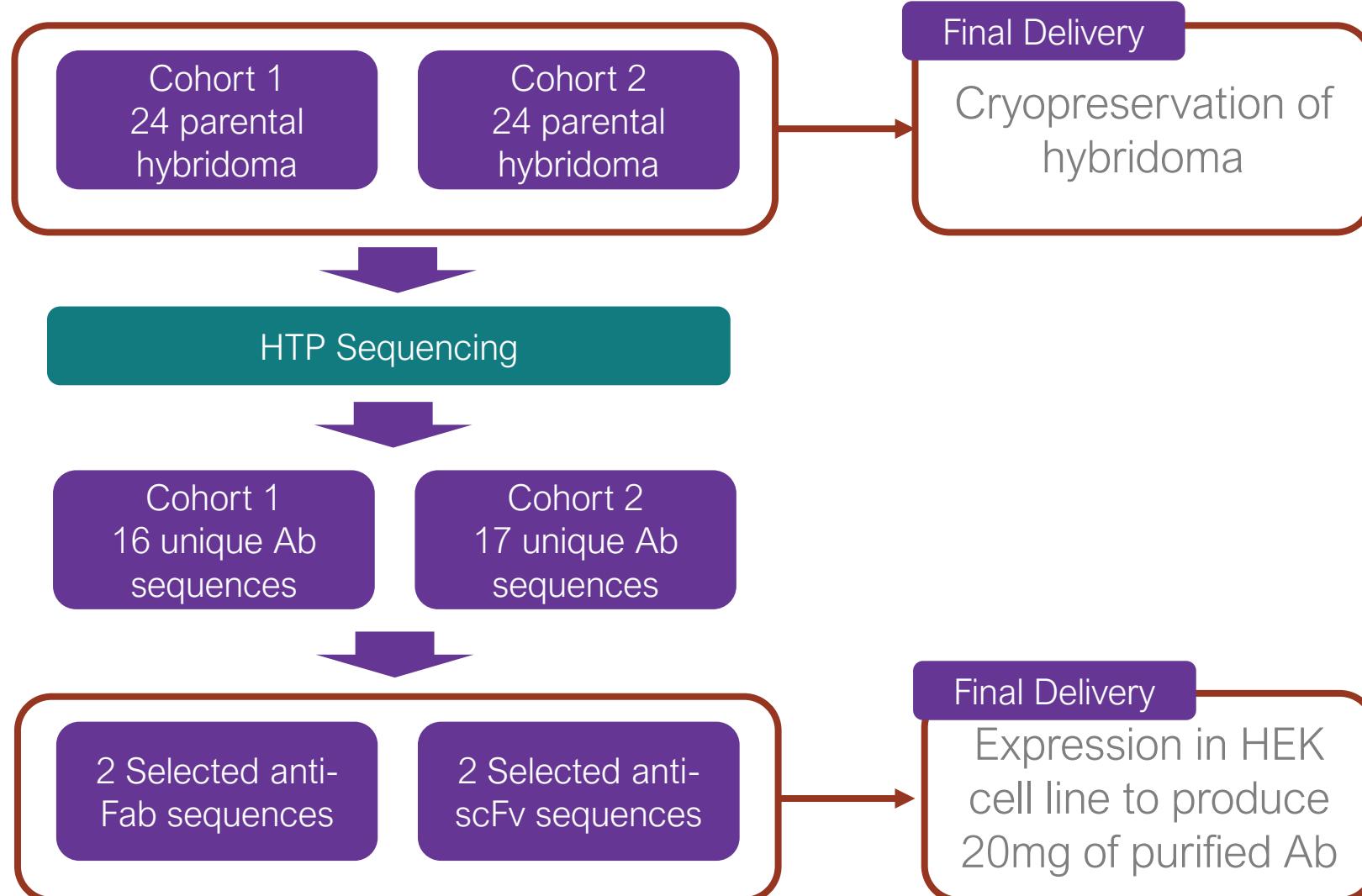
◆ Ligand



*All Cohort 2 anti-svFc antibodies are neutralizers

DELIVERY PROJECTS AS SEQUENCES AND PURIFIED ANTIBODIES

Direct HTP sequencing on parental hybridoma clones for efficient recovery of antibody HC/LC pairing



Conclusions

- Rapidly generated mAbs against the ScFv and Fab arms of a bi-specific with the desired characteristics:
 - Highly specific, including in the presence of human serum
 - mAbs that work well as pairs
 - Blocking and non-blocking profiles identified
 - Diverse affinities
- Delivered:
 - HC/LC sequences
 - Purified and validated mAb
 - pcDNA3.4 vector
- Delivered final product 1 week ahead of pre-determined schedule