

A decorative pattern of light blue antibody icons arranged in a grid on the left side of the slide. The icons are stylized Y-shapes with rounded ends, representing antibody molecules.

Case Study 2

Ultrafast Single B Cell-based Anti-idiotypic mAb
Discovery against an mAb Therapeutics

RAPID, B CELL-BASED ANTI-IDIOTYPIC ANTIBODY DISCOVERY

Delivering PK assay-ready mAbs in 11 weeks to meet aggressive clinical timelines

Applications and Background

- Development of assay-ready mAbs for a PK assay for a therapeutic antibody (IgG) going into trials very soon
- Partner failed a discovery campaign internally (poor titer) and brought in Abveris help to avoid delaying clinical trials



Urgent delivery required
(< 3 months)

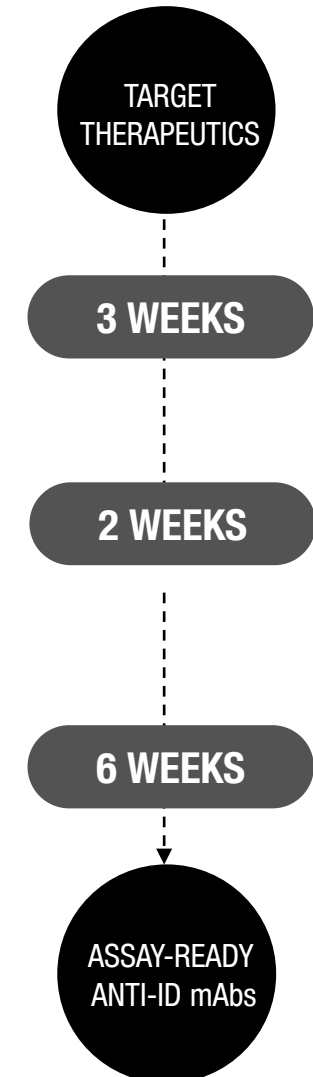
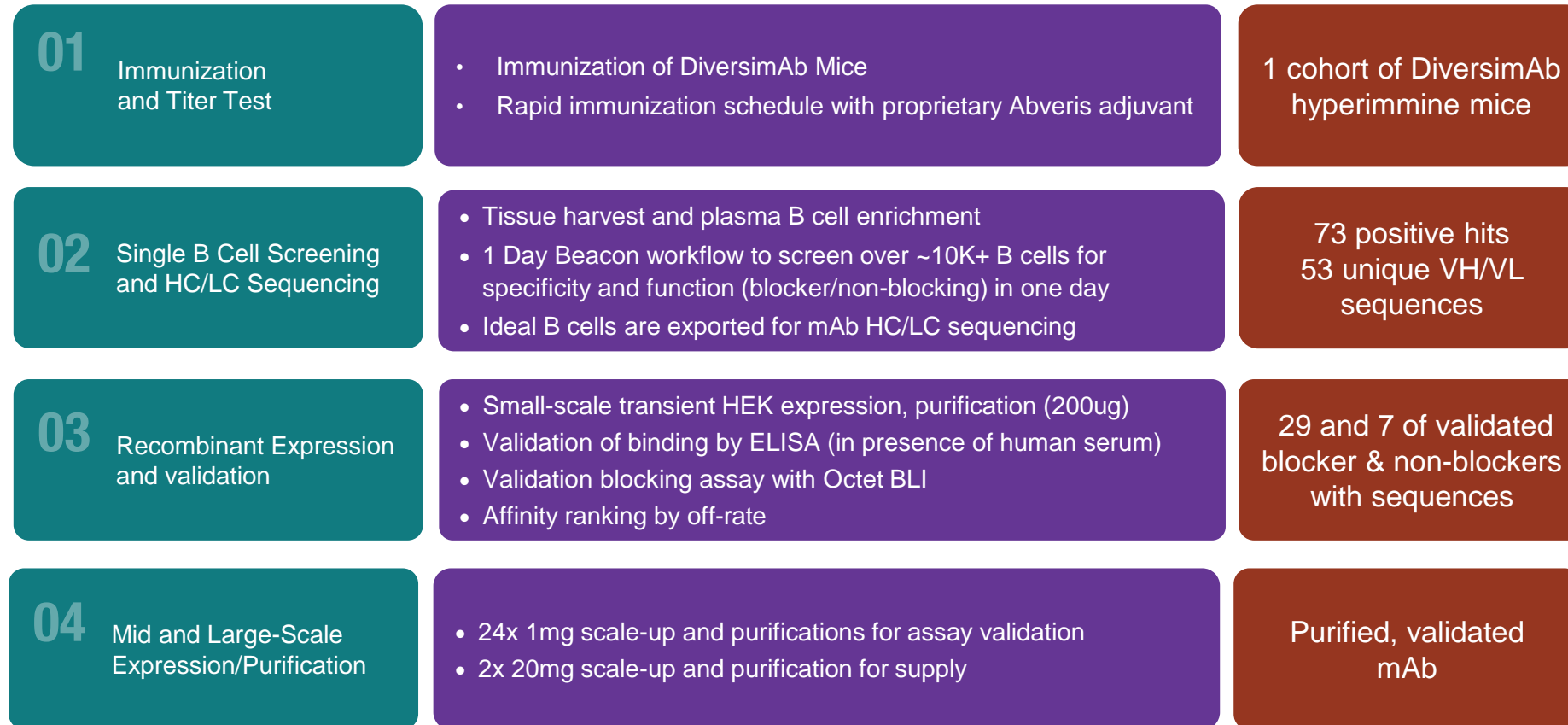


Requirement and Requested Deliverables

- High specificity, no off-target binding for clean PK assay results
- Blocking and non-blocking mAbs required
- A range of affinities
- Antibody sequences and validated small-scale, purified antibodies now. Larger quantities of mAb soon after.
- ASAP timelines (less than 3 months)

ANTI-IDIOTYPIC ANTIBODIES DISCOVERY- ULTRAFAST SINGLE B CELL WORKFLOW

Delivering PK assay-ready mAbs in 11 weeks to meet aggressive clinical timelines



BEACON-BASED SINGLE CELL ASSAYS FOR BINDING PROFILE

Multiplex, sequential assays for (1) specificity to Target Fab, with no reactivity to (-) control antibody and (2) ligand blocking assay



Assay 1: Specificity Assay	Assay 2: Blocking Assay
Goat anti-Mouse Fc beads + -Off-Target Control mAb-AF488 (600nM) + Goat anti-Mouse Fc AF647 (1:500)	Goat anti-Mouse Fc beads + Target Fab-AF488 (200nM) + Ligand-AF647 (400nM)

IgG secreting B cells screened = 10,784

AF647 **AF488** **AF647** **AF488**

Hit Count

50

23

Target-specific binder, Ligand non-blocker	+	-	+	+
Target-specific binder, Ligand blocker	+	-	-	+
Target-specific binder, partial-blocker (or low affinity binder)	+	-	-	-
Nonspecific binder	+	+	+ or -	+ or -
Non-binder	+	-	-	-

Legend

- Live plasma B cell
- B cell secreted Ab
- Goat anti-mouse Fc-AF647 (detection Ab)
- Goat anti-Mouse Fc beads
- Off-target Control
- Target Fab-AF488
- Ligand-AF647

BEACON-BASED SINGLE CELL ASSAYS FOR BINDING PROFILE

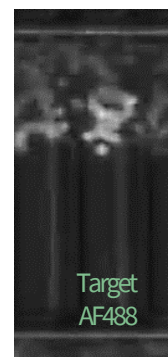
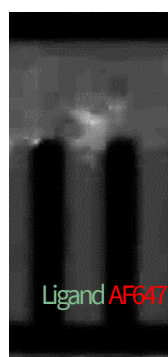
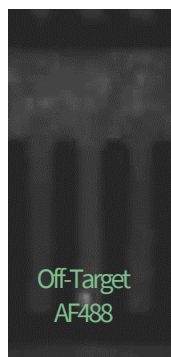
Multiplex, sequential assays for (1) specificity to Target Fab, with no reactivity to (-) control antibody and (2) ligand blocking assay



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AF647 AF488 AF647 AF488

Target-specific binder, Ligand non-blocker	+	-	+	+
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BEACON-BASED SINGLE CELL ASSAYS FOR BINDING PROFILE

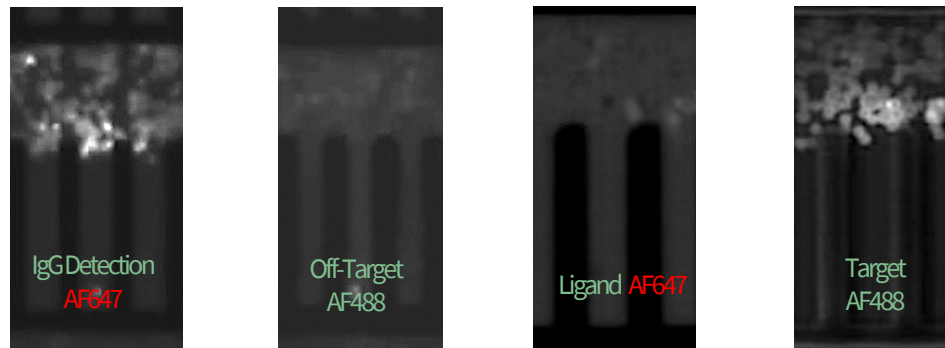
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AF647 AF488 AF647 AF488

Target-specific binder, Ligand blocker	+	-	-	+



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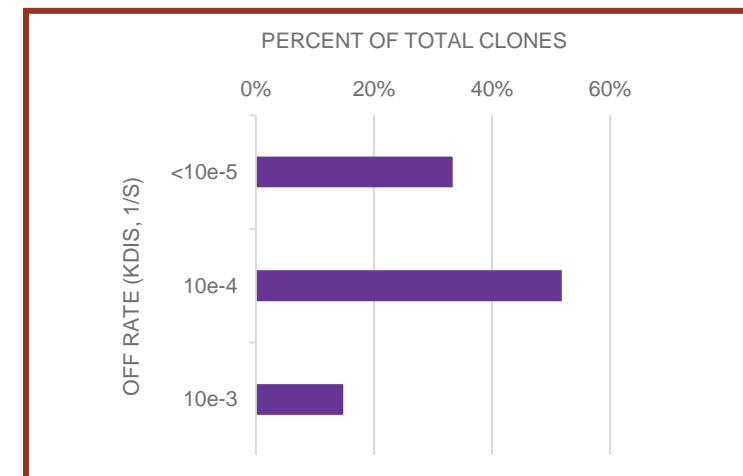
ANTIBODY EXPRESSION AND DOWNSGREAM VALIDATION

Antibodies were expressed and further validated by ELISA and Octet for binding profile

Number of Antibodies	1 Hits exported for sequencing	2 Unique sequences obtained	3 Small scale expression	4 ELISA validated	5 Blocking assay by Octet BLI
	Blocker	50	37	33	36
Non-Blocker	23	16	14		7



Affinity Ranking of 36 Octet BLI validated antibodies



3 Small-Scale Expression

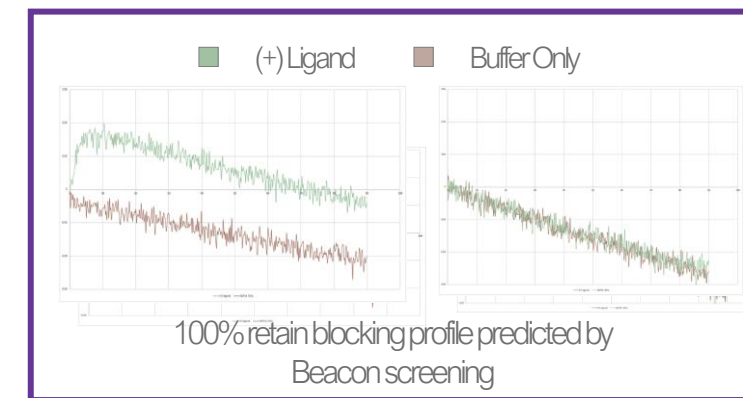
Recombinant expression
89% efficiency by Octet validation (confirmed expression)
Small-scale high throughput Expi293 expression and purification:
Average 225 µg ± 60 µg yield

4 ELISA Validation in the Presence of Human Serum

Clone	Target Fab (+)	Target Fab in 20% NHS (+)	Target IgG (+)	Irrelevant Fab-His (-)	Poly HulgG (-)
1	1.0868	0.824	1.0647	0.0572	0.0598
2	1.0036	0.3936	1.0998	0.0715	0.13
3	0.9802	0.4544	0.9594	0.0585	0.0624
4	0.9243	0.3792	0.8541	0.0559	0.0585
5	0.8879	0.1776	0.9711	0.0572	0.0663
6	0.8762	0.2576	0.9425	0.0559	0.0598
7	0.6188	0.144	0.6279	0.0598	0.3588

ELISA screening cascade including matrix using 20% human serum, 77% retain activity in the presence of human serum
7 example clones shown

5 Octet Blocking Assay for activity validation



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Conclusions

- 11 weeks from the start of immunization to delivery of validated small-scale, purified antibodies with sequences
- High quality mAbs delivered – specific, high affinity mAbs with blocking and non-blocking profiles
- Partner was able to develop highly sensitive PK assay and move clinical trials forward on time