APPLICATION NOTE

Rapid High-Resolution Screening of Plasma B Cells to Identify Antibody Lead Candidates

APPLICATION SPOTLIGHT

- Deliver anti-idiotypic antibodies with competitive ligand-blocking activity in half the time of a traditional hybridoma screen
- Discover a diverse panel of rare, high-affinity antibodies that could not be discovered using phage display against an antigen with a completely homologous off-target product
- Develop an adherent cell-based binding assay that enables discovery of lead candidate sequences against a challenging membrane-bound target in just 29 days

INTRODUCTION

Biotherapeutics comprise a large portion of the treatment options for a wide range of conditions and account for almost half of recent drug approvals[1]. The first wave of antibody therapeutics appeared in the clinic relatively quickly since their biological functions and disease correlations were well understood[2]. However, the pace of new biologic development has decreased over time because many potential new targets are membrane proteins with complex structures and biological functions. To keep up with the demand for new therapeutics, biopharmaceutical companies need to incorporate enhanced methods and technologies to generate antibodies against these difficult targets to accelerate their drug development timeline.

The Opto™ Plasma B Discovery workflow on the Beacon® system enables high-resolution screening on plasma B cells with unprecedented speed to generate diverse hit panels and to select antibody lead candidates for rare epitopes and difficult targets (Figure 1). Flexible assay

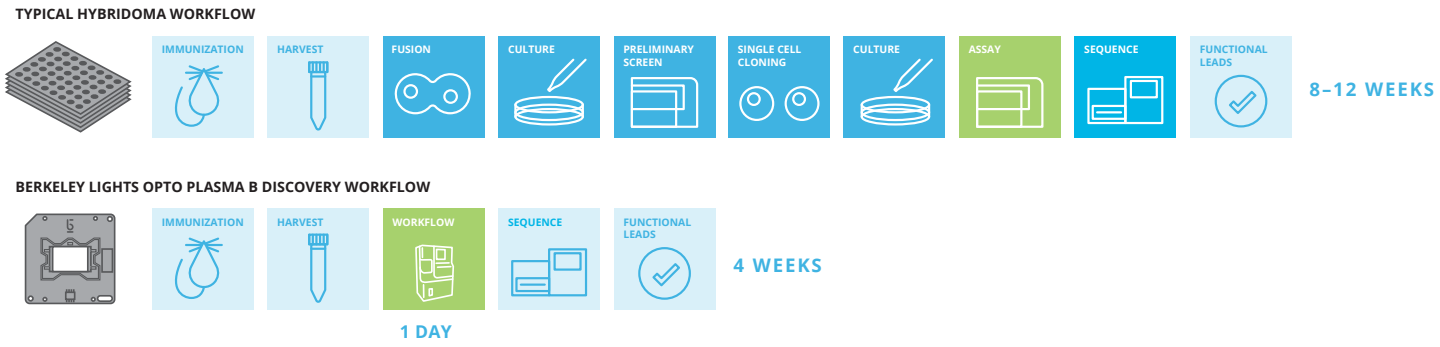

Figure 1. The Opto Plasma B workflow decreases screening and lead candidate selection time from 8–12 weeks to less than 4 weeks. Antibodies can be functionally screened in a single day using both bead- and cell-based assays, enabling functional characterization during primary screening.

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formats and the ability to run sequential bead-based and cell-based assays on the same B cell gives users the ability to rapidly characterize and down-select potential lead candidates against soluble and membrane target antigens.

In this application note, we share three case studies demonstrating how Abveris, a monoclonal antibody discovery partner, has used the Opto Plasma B Discovery workflow to perform high-throughput, high-resolution screening of plasma B cells to rapidly select antibody lead candidates. We highlight how Abveris has successfully developed adherent cell-based assays for the discovery of antibodies against challenging targets that can neither be expressed in soluble form nor in suspension cells. These capabilities all contribute to driving development costs down and increasing the speed at which new therapeutics can be introduced to the clinic.

MATERIALS AND METHODS

Mouse immunization and B-cell enrichment

Mouse immunizations were performed at Abveris in accordance with all IACUC protocols. Eight- to twelve-week-old DiversimAb™ hyperimmune mice received immunizations following a RIMMS schedule at subcutaneous sites using a custom adjuvant formulation reconstituted in an oil-in-water emersion over the course of 18 days. Target antigenic proteins were provided by partner companies (undisclosed) and used at a dosage ranging from 10-100 µg per injection. The mice received a final boost 3 days before harvesting lymph nodes. From a B cell enriched population, plasma B cells were isolated using a magnetic plasma cell isolation kit. Isolated B cells were resuspended at the BLI-recommended density and stained for targeted penning.

Opto Plasma B Discovery workflows on the Beacon system

Plasma B cells were imported onto OptoSelect™ 14k chips following a modified Opto Plasma B Discovery 1.0 workflow. Briefly, through a customized Targeted Pen and Selection (TPS) penning algorithm, live IgG-secreting plasma B cells were penned via OEP™ technology as single cells over multiple rounds of imports. Pens containing multiple cells were unpenned. Cells were penned in a custom media formulation containing Cell MAb Medium Quantum Yield (ThermoFisher Scientific) supplemented with FBS, growth factors, and Loading Reagent (Berkeley Lights). Assay and culture conditions utilized the same media without Loading Reagent.

Bead-based screening assays on the Beacon system

Prior to Beacon workflows, recombinant proteins were labeled at primary amine sites with AF488 or AF647 using Alex Fluor 488 TFP Ester or Alexa Fluor 647 NHS Ester, respectively (ThermoFisher Scientific). Biotinylated proteins for Streptavidin bead-based screens were supplied by partner companies (undisclosed). For affinity screening and ligand blocking assays, chips containing penned plasma B cells were loaded with single or multiplexed assay mixtures composed of anti-mouse IgG coated beads (Spherotech) and fluorescently labeled target protein(s) at optimized concentrations. Blocking assays were performed with multiplexed detection for both fluorescently labeled (AF488) target receptor and the cognate ligand (AF647 labeled). High specificity screening assays were performed by loading chips with anti-mouse IgG AF488-labeled secondary antibody (Jackson ImmunoResearch) and Streptavidin coated beads (Spherotech) that were pre-incubated with biotinylated target protein and washed in PBS prior to import. With all assay formats, secreted antibody from plasma B cells diffused from the NanoPen™ chambers into the channel where they bound the beads and concentrated fluorescently labeled protein(s) or secondary antibodies, forming fluorescent halos (“blooms”) in the channels adjacent to the pens containing antigen-specific plasma cells. The chips were imaged using a FITC and/or Cy5 filter cube. For blocking assays, blooms detected in both the FITC and Cy5 channels indicated non-blocking antibody secretion, while blooms detected in only the FITC channel indicated blocking antibody secretion. If sequential assays were required, the bead assay mixture was flushed from the chip before loading a second assay mixture. All assays were scored with human verification.

Cell-based screening assays on the Beacon system

Chips containing penned plasma B cells were loaded with single assay mixtures consisting of anti-mouse IgG AF647-labeled secondary antibody (Jackson ImmunoResearch) and cell lines overexpressing the target of interest or parental cells lines as a control. Prior to loading, cell lines were harvested and equilibrated in import buffer at optimal densities. Secreted antibodies capable of binding

the transmembrane protein presented on the cell surface were detected as blooms in the Cy5 channel. If sequential assays were required, the cell assay mixture was flushed from the chip before loading a second assay mixture. All assays were scored with human verification.

Sequence recovery and recombinant expression
Cells of interest were selected for single cell export into 96-well PCR plates containing lysis buffer. Paired heavy and light chain sequences were amplified using gene-specific primers for cDNA production and two successive rounds of nested PCR. Nucleotide sequence from amplicons was determined by Sanger sequencing in reverse orientation and data was analyzed in Geneious Biologics for antibody annotation using the Single Clone Antibody Analysis pipeline. Paired heavy and light chain sequences were codon optimized, synthesized (ThermoFisher Scientific), cloned into separate mammalian expression vectors and co-transfected into Expi293 cells. Antibody expression in saturated supernatant was confirmed by Bio-Layer Interferometry (Sartorius), purified by Protein G and quantitated by UV absorption spectrophotometry.

Downstream characterization of lead candidates
To validate off-chip binding and activity to target proteins, plate-based ELISA measurements were performed along with Bio-Layer Interferometry (Sartorius) and surface plasmon resonance (Carterra). To assess specificity, ELISA assays were conducted in a titration series of the experimental antibody at a starting concentration of 20 µM with target or off-target control protein immobilized on the plate surface, both in the presence or absence of a defined matrix interference buffer. Affinity assays were performed on the Octet RED96e (Sartorius) or Carterra IBIS MX96 platforms using anti-mouse Fc biosensors or CMD50 sensor chips, respectively, to immobilize experimental antibodies. Association measurements were acquired using multiple concentrations of target or off-target control proteins followed by dissociation measurements in buffer alone.

For competitive blocking assays, a classical sandwich format (sequential binding) was utilized for the Octet system and a pre-complexed format for Carterra. In the sequential binding format, antibody was immobilized using anti-mouse Fc biosensors, followed by saturation with target receptor. The captured antibody-receptor complex was assessed in various concentrations of ligand to determine the ability to form an antibody-receptor-ligand complex. Antibodies capable of capturing receptor and preventing the subsequent antibody-receptor-ligand complex formation were classified as blocking. A negative control condition was assessed for comparison where the antibody-receptor complex was assessed in buffer alone to obtain a baseline measurement of dissociation rate. Antibody-ligand interactions were also assessed to eliminate the possibility of non-specific binding. In the pre-complexed format, target receptor and ligand were premixed at a fixed concentration of receptor and various concentrations of ligand before association was measured to antibody immobilized on the chip surface. The association rate of the receptor-ligand complex was compared to that of the receptor alone at a matched concentration. Antibodies with negligible binding to the receptor-ligand complex at high molar excess concentrations of ligand were deemed competitive binders.

RESULTS AND DISCUSSION

Case Study 1: Rapid discovery of anti-idiotypic antibodies with target-blocking activity
In this case study, there was an urgent need to rapidly discover anti-idiotypic antibodies for PK/ADA assay development due to extremely tight timelines for a clinical trial. Abveris performed a custom rapid immunization protocol using their DiversimAb hyperimmune mouse model system and subsequently screened B cells generated by these mice using two different Opto Plasma B Discovery assays in just 1 day to identify B cells that produced IgGs capable of blocking the ligand binding interaction. Lead candidates were exported, sequenced, and recombinantly-expressed as IgG molecules to validate binding and function before a panel of highly specific, high-affinity candidates were delivered to the partner. The project required only 9 weeks to complete, from the start of immunization to antibody delivery, compared to the approximately 18 weeks for a side-by-side traditional hybridoma approach, cutting lead discovery time in half.

Plasma B cells isolated from the immunized mice were screened on the Beacon system for antigen specificity, cross-reactivity, and functional ligand blocking activity. Over 10,000 single plasma B cells were loaded onto each OptoSelect chip with single B cells captured in NanoPen chambers. Anti-IgG capture beads were then loaded onto the chip with fluorescently-labeled antigen at a low concentration optimized to identify cells that secreted

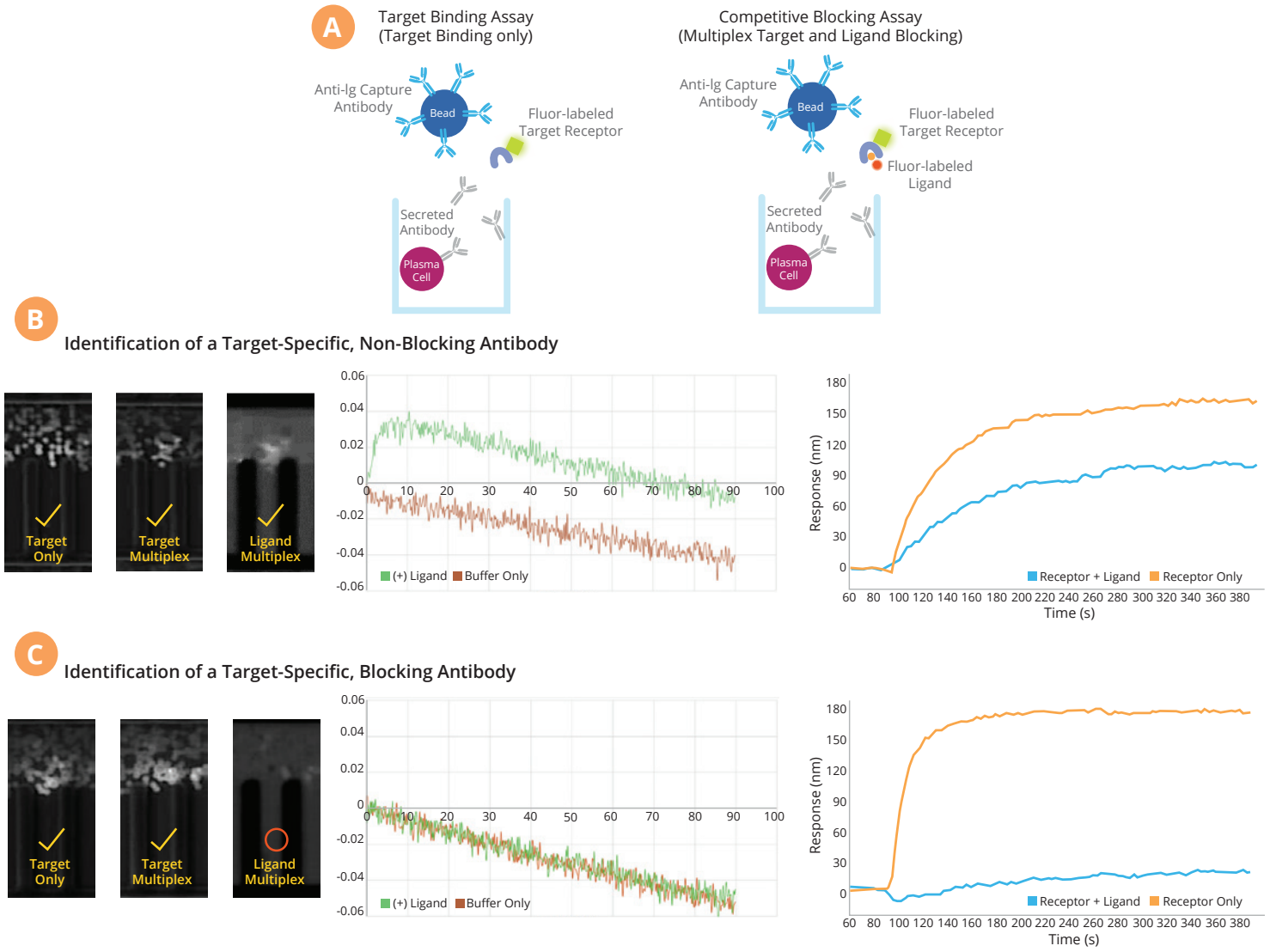

Figure 2. Identification of anti-idiotypic antibodies with competitive ligand blocking activity. A. Schematic of assays performed during Opto Plasma B Discovery workflows on the Beacon system to screen single B cells for target binding and competitive ligand blocking activity in just 1 day. **B** and **C**. Lead candidates were then exported, sequenced, cloned, re-expressed, and purified for further downstream analysis. Beacon (left), Octet (middle), and Carterra (right) systems were used to identify and characterize target-specific, non-blocking **(B)** and blocking **(C)** antibodies.

antibodies with high affinity for the antigen (Figure 2A). After completion of the binding assay, the reagents were flushed from the channels and anti-IgG capture beads with a fluorescently-labeled antigen mixed with fluorescently labeled ligand were loaded to perform a competitive ligand-blocking assay to further down-select lead candidates (Figure 2A). In a single day, Abveris was able to identify >200 cells secreting antibodies with binding and desired blocking activity, significantly decreasing the development time due to the speed of the initial screen as well as the advantage of capturing higher-quality candidates earlier in the screening process.

During characterization of recombinantly-expressed lead candidates, 82% demonstrated confirmed binding activity. All of the candidates with confirmed binding activity retained blocking activity profiles identified by Beacon screening (Figure 2B and 2C).

A similar number of lead candidates were identified when plasma B cells from the same mice were screened using the Opto Plasma B Discovery workflow and traditional hybridoma technology. However, the hybridoma screening process took approximately 4–6 weeks, compared to the 1 day on the Beacon system. Additionally, antibodies

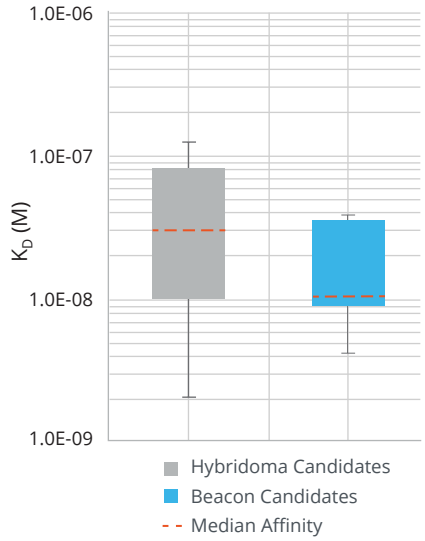

Figure 3. Affinity comparison confirms that higher-affinity candidates are identified using the Beacon system compared to a traditional hybridoma screen. Affinities in the low double or single nanomolar range were observed for candidates identified using the Beacon. In comparison, a larger range of affinities was observed with the candidates identified by traditional hybridoma screening with a median affinity approximately 3x lower than that of candidates identified on the Beacon system.

obtained from the Beacon system exhibited binding affinities that were, on average, approximately 3x higher than those of antibodies identified using the hybridoma screen (Figure 3). Identifying high-affinity candidates is possible using traditional hybridoma screens, but required more work downstream to separate the lower-affinity candidates from the higher-affinity candidates.

Case Study 2: High-resolution screening identifies rare antibodies with specificity to target over a high-homology off-target product

Identifying a specific antibody to a target can be challenging if that target is highly homologous to an off-target protein. In this case study, Abveris was searching for candidates that would selectively recognize the monomeric form of a serum protein, and not its completely-homologous tetrameric form (Figure 4). The oligomeric form of the protein is present in human serum at high micromolar concentrations, requiring an antibody without any cross-reactivity to the oligomer. A previous attempt using phage display only yielded two antibodies of interest; one with poor developability and one with poor affinity.

Abveris was able to screen a highly diverse plasma B cell repertoire using the Beacon system to identify rare antibodies without any off-target cross-reactivity. Plasma B cells from immunized DiversimAb mice were loaded into individual NanoPen chambers on an OptoSelect chip before performing a binding assay for monomer specificity by importing an assay mixture into the chip containing streptavidin capture beads pre-complexed to biotinylated monomeric antigen and fluorescently labeled detection antibody. The monomer binding assay reagents were washed out of the chip before streptavidin capture beads pre-complexed to biotinylated tetrameric antigen were loaded onto the chip to perform the assay for tetramer binding activity. The ability to perform sequential assays on the same cells and in the same assay format enabled identification of unwanted cross-reactive antibodies with very high sensitivity.

Of >15,000 B-cells screened, nearly two thousand clones were discovered that bound to both the monomeric and oligomeric forms of the protein. However, only 146 highly specific plasma B cells secreting antibodies were identified that recognized the monomer and not the tetramer (Figure 5A). Plasma B cells secreting these monomer-specific antibodies were exported from chips

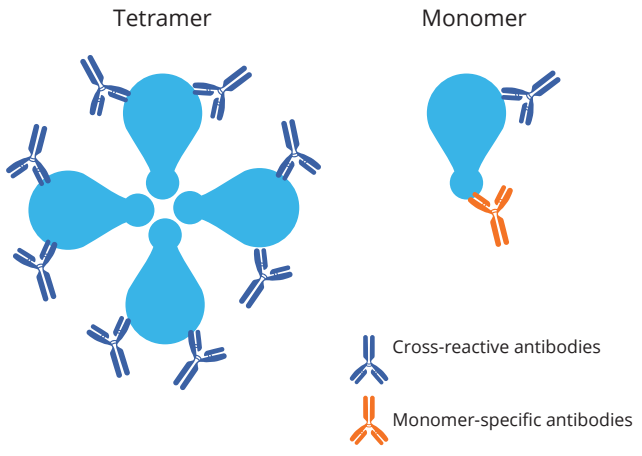

Figure 4. Identification of monomer-specific antibodies requires high-resolution screening against both target monomer and its completely-homologous tetrameric form.

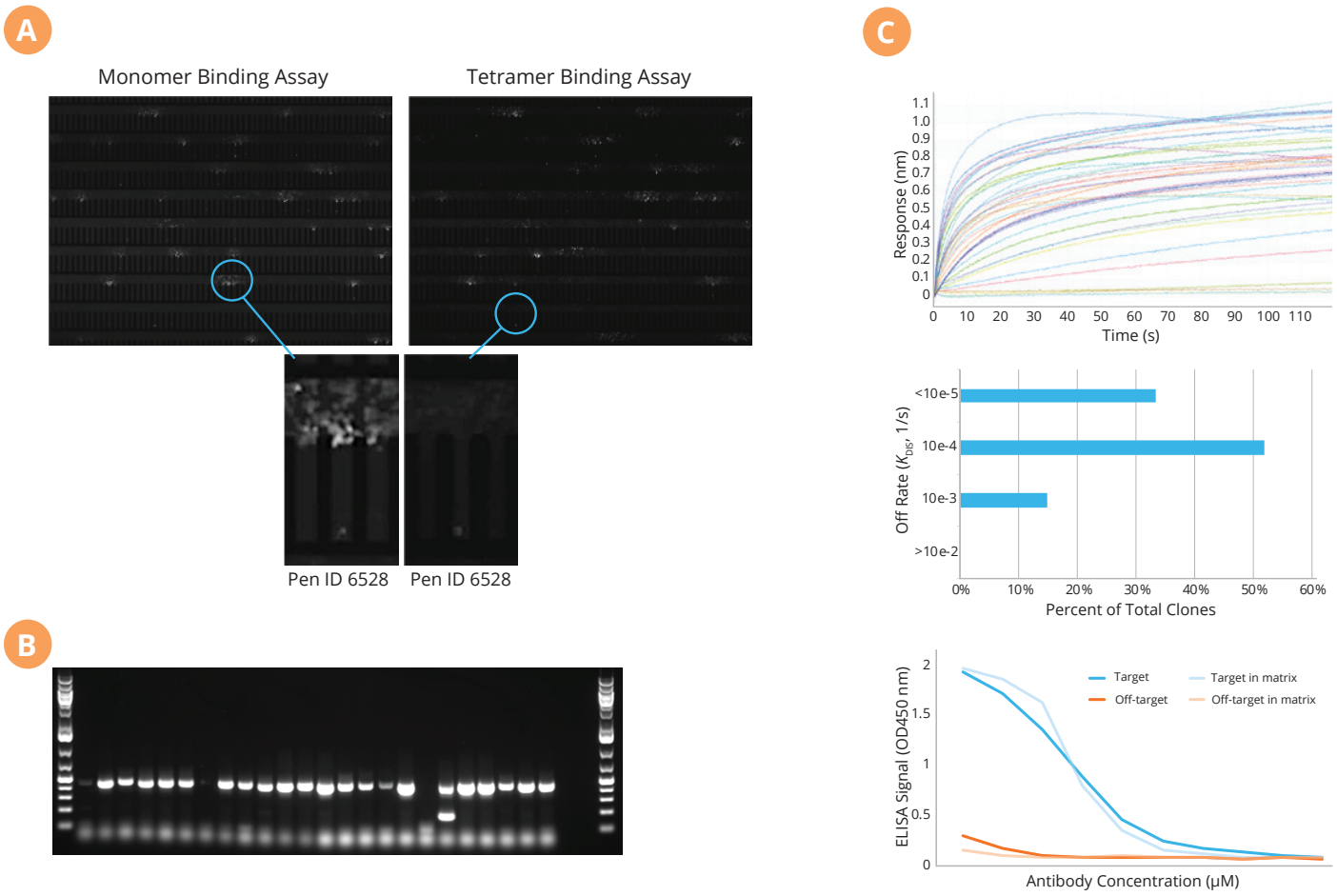

Figure 5. Identification of rare antibodies with specificity to the monomeric form of a serum protein over its completely-homologous tetrameric form. A. Single plasma B cells were screened to identify candidates that recognized the target monomer without cross-reactivity to the completely homologous tetramer. **B.** Plasma B cells secreting lead antibody candidates were exported and sequenced with up to a 70% heavy/light chain recovery rate as measured by gel electrophoresis. **C.** 36 of the antibodies were further analyzed with the Octet system and ELISA to confirm IgG expression (top), affinity (middle) and binding specificity (bottom). Most of the antibodies recognized only the target antigen with low single-digit nanomolar affinities (off-rate data shown here) and were well-behaved in the serum matrix.

to recover their paired heavy/light chain sequences and 36 were selected for recombinant expression (Figure 5B). These antibodies were purified with high yield for further affinity characterization using the Octet system and monomer specificity analysis by ELISA (Figure 5C). The top candidates exhibited low single-digit nanomolar affinity for the monomer, with no detectable binding to the oligomer even in the presence of a serum matrix (i.e. buffer with high serum concentration).

This case study showcases how the Beacon system enables the screening of a massive panel of antibodies to identify ideal, unique, and highly specific candidates. Using the Opto Plasma B Discovery workflow, Abveris was able to deliver a panel of high-affinity antibodies that behaved well in a serum matrix. Additionally, the ability to avoid an excessively large hybridoma campaign to identify these rare antibodies translated to significant cost and time savings for this program.

Case Study 3: Development of adherent cell-based assays to select lead candidates against difficult membrane-bound targets

In a final case study, Abveris demonstrated the rapid generation of a panel of antibodies against a cell surface receptor that is expressed only on adherent cells. Abveris used the flexibility of assays on the Beacon system to develop and optimize a cell-based assay using adherent cells for a project with a short timeline that precluded a hybridoma campaign.

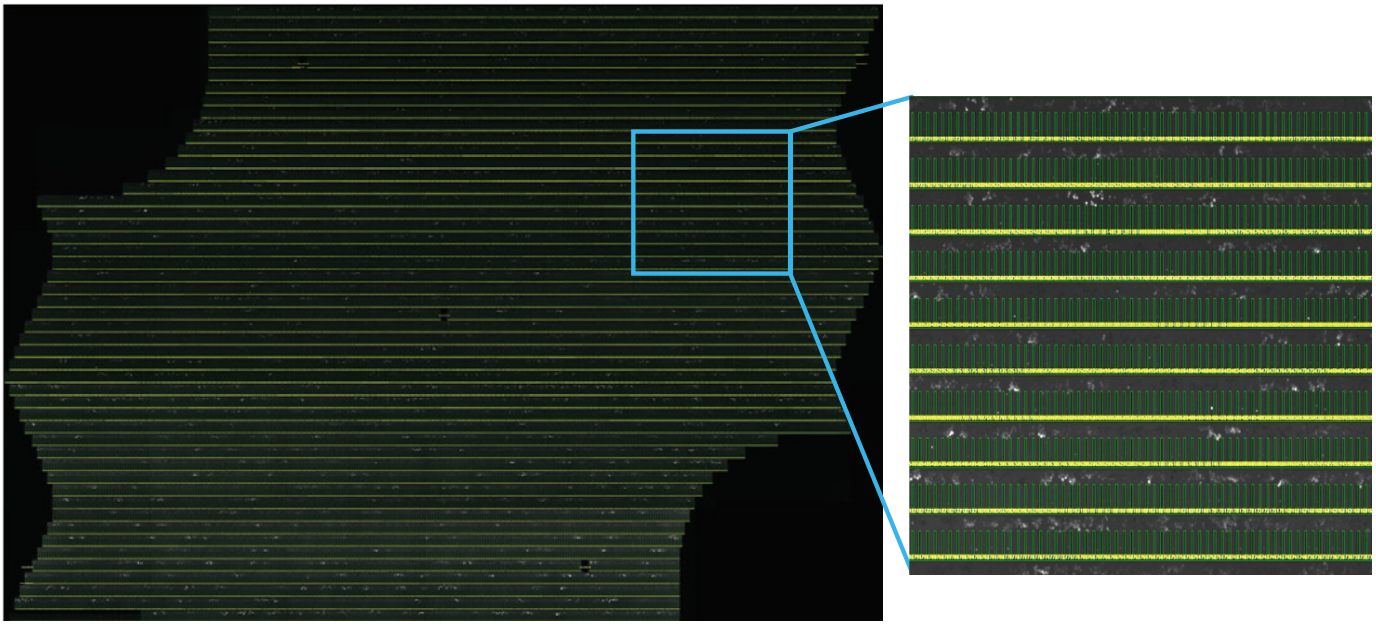

Figure 6. A cell-based binding assay using antigen-expressing adherent cells. Zooming into a portion of the OptoSelect chip demonstrates how plasma B cells secreting antibodies that recognize the antigen expressed on an adherent HEK cell line can be identified.

A cell-based screening assay was developed during which single B cells were loaded into NanoPen chambers and isolated adherent HEK cells were loaded onto the chip along with a fluorescently labeled detection antibody to identify secreted antibodies that recognized the receptor expressed on the cell surface. Pre-processing of the adherent HEK cells to a dense single cell suspension facilitated an even cell distribution, while chip import conditions and buffer optimization enabled the cells to remain stable over the course of the assay and enabled them to be effectively flushed from the chip upon assay completion. The assay optimization conditions resulted in better assay performance for the accurate identification of promising lead candidates (Figure 6).

Using this approach, Abveris was able to identify over 1,000 plasma B cells that secreted antibodies specific to the target receptor expressed on HEK cells and without cross-reactivity to a negative-control parental HEK cell line assayed in a sequential format. 192 plasma B cells were exported with greater than 60% paired heavy/light chain sequence recovery and this campaign was completed in just 29 days from immunization to delivery of sequences to Abveris' partner. As a result of this rapid turnaround, the partner has initiated three additional campaigns to similar target classes, two of which have been completed to date and have shown similar success rates.

CONCLUSION

The Opto Plasma B Discovery workflow on the Beacon system gives users the speed, throughput, screening resolution, and flexibility to innovate that is required to keep up with the demand for new biotherapeutics. In this application note, Abveris has demonstrated how the Beacon system can be used in conjunction with their DiversimAb hyperimmune mouse model to accelerate the discovery and selection of rare lead candidates against difficult targets.

Abveris was able to identify high-quality leads, as demonstrated by the high percentage of candidates with confirmed binding and functional activity, in half the time it takes using traditional hybridoma technology. The high-resolution screening of the Opto Plasma B Discovery workflow also allowed Abveris to identify rare, high-affinity antibodies with specificity to the monomeric form of a target without cross-reactivity to its tetrameric form, when

an *in vitro* phage display approach was unable to isolate one antibody with the characteristics required to develop an efficacious treatment. Finally, the flexibility offered by the Beacon system empowered Abveris to develop an assay to select lead candidates against a difficult membrane target by screening plasma B cells using an adherent HEK cell line expressing the receptor antigen.

As the targets for potential new biotherapeutics become more complex, the technologies that support antibody discovery must keep pace. The Opto Plasma B Discovery workflow on the Beacon system gives users the ability to identify better lead candidates for difficult targets faster, all while saving costs during discovery and downstream characterization.

ABOUT ABVERIS

abveris

Abveris is an antibody discovery partner specializing in the discovery of therapeutic antibodies against challenging target classes. Abveris applies advanced immunization methods combined with B cell screening to provide gene-to-antibody discovery services. Abveris is developing the next generation of biologics, cell therapies, vaccines, and diagnostics in partnership with global leaders in biopharma. Additional information about Abveris is available at: www.ABVERIS.com.

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