Rapidly Discover and Select Potent Ligand/ Receptor Blocking Antibody Lead Candidates with Opto™ Plasma B Discovery

APPLICATION SPOTLIGHT

- Maximize hit recovery and down-select blocking antibodies from plasma B cells in a single day
- Access greater diversity by screening B cells from multiple organs, such as bone marrow and spleen
- Rapidly down-select lead candidates with confirmed binding and blocking activity in plate-based assays

INTRODUCTION

Over the past three decades, antibody therapies have been developed for a host of different diseases, ranging from autoimmune disorders to infectious diseases and cancer. Though traditional antibody discovery technologies have been successful when screening against simple targets, there has been limited success against difficult membrane targets such as GPCRs and ion channels due, in part, to the difficulty in recombinantly expressing these proteins in their native conformation and screening enough of the immune repertoire to identify the rare target specific antibodies. Antibodies screened against recombinant antigens are often not viable drug candidates because they do not have the desired antibody specificity or functional potency in *in vivo* models. Therefore, cell-based assays may enable screening against native antigens and, therefore, may accelerate therapeutic antibody lead candidate selection.

Opto Plasma B Discovery on the Beacon® optofluidic system screens plasma B cells for antigen-specific hits and down-selects lead candidates in a single day (Figure 1) using multiple assays for antigen specificity and function against both recombinant and cell-surface antigens (Figure 2). This comprehensive screening paradigm, coupled with early functional evaluation accelerates selection of lead antibody candidates to difficult targets early in the drug discovery process, which translates to reduced time and expense incurred when irrelevant, non-functional hits are sequenced or cloned.

The blocking of receptor-ligand interactions has been found to be an effective therapeutic approach for many diseases, including cancer. For example, in normal cells, the interaction between the receptor PD-1 and its ligand PD-L1 acts as an immune checkpoint to guard

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against autoimmunity. However, some cancer cells have hijacked this interaction to evade immune surveillance. Commercially available PD-1 and PD-L1 inhibitors like atezolizumab, durvalumab, and avelumab are approved against bladder cancer, non-small cell lung cancer, and metastatic Merkel cell carcinoma and represent a class of cancer immunotherapy that has changed the landscape of oncology.

In this application note, we demonstrate how Opto Plasma B Discovery enables the rapid selection of receptor-ligand blocking antibodies using the therapeutically-relevant PD-1/PD-L1 model system. We also show that by screening not only primary single B cells from the spleen but also those from bone marrow, we were able to discover more blocking antibodies and identify unique candidates that may be missed using traditional hybridoma technology.

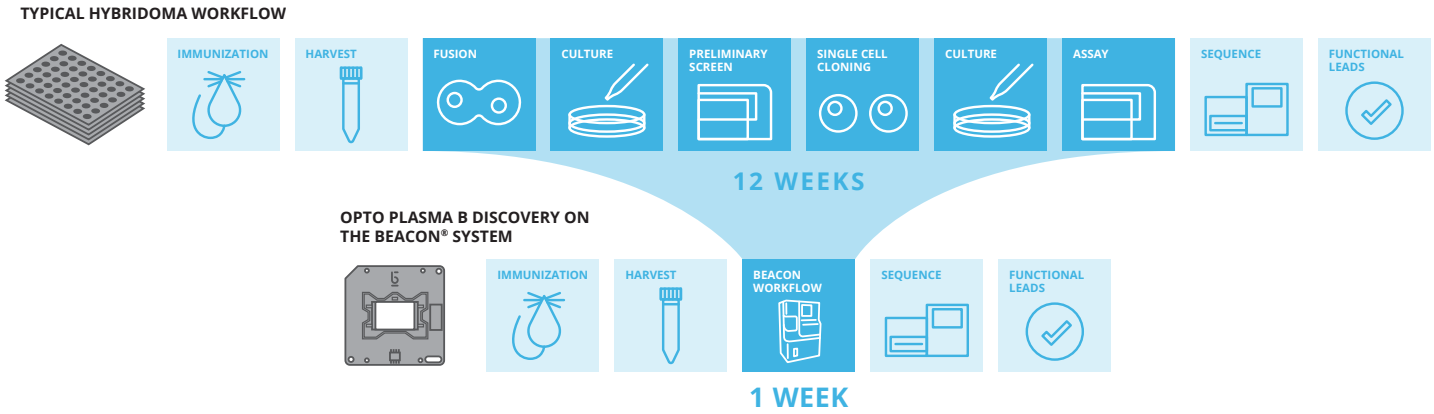


Figure 1. Rapid screening and selection of antibody lead candidates directly from plasma B cells without time-consuming and inefficient hybridoma generation.

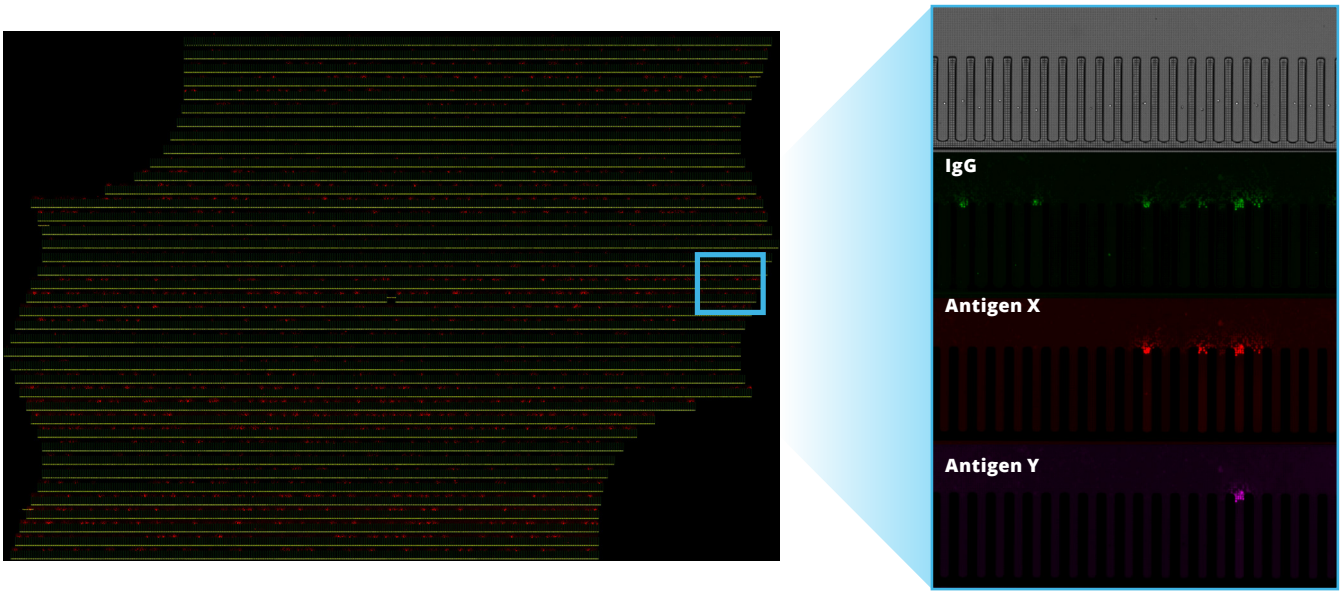


Figure 2. Rapid identification of antigen-specific and cross-reactive antibodies from plasma B cells. Three assays were multiplexed for simultaneous fluorescence measurements of IgG secretion (**green**), binding to antigen X (**red**) and binding to antigen Y (**magenta**). Multiple assays enable the down-selection of lead candidates prior to sequencing, cloning , re-expression, and purification.

MATERIALS AND METHODS

Cell preparation and assay reagents

We isolated primary plasma cells from the bone marrow and spleen of Balb/c mice immunized with Fc-fused PD-L1 extracellular domain (huPD-L1 ECD-FC) using a CD138+ plasma cell isolation kit (Miltenyi Biotech). We then prepared PD-1-AF488 by labeling a recombinant PD-1-Fc fusion protein (ChemPartner) using an AF488 labeling kit (Thermo Fisher Scientific). We prepared recombinant PD-L1 beads by coupling biotinylated PD-L1 (ChemPartner) to streptavidin polystyrene particles (Spherotech Inc.). Finally, CHO-K1 cells were engineered to over-express human PD-L1 (ChemPartner).

Antibody screening assays

Single plasma cells were loaded into individual NanoPen™ chambers on OptoSelect™ 11k chips using Bruker Cellular Analysis OEP™ technology. CHO-K1-PD-L1 cells were then bulk loaded into individual NanoPen chambers so that an average of 4 cells were loaded per pen. We then loaded an assay mixture of antigen-coated beads and secondary antibody to simultaneously perform a recombinant PD-L1 bead binding assay (in-channel) and cell binding assay (in-pen). The assay mixture was then flushed out of the chip to perform the ligand/receptor-blocking assay. Cell-based assays were scored by human verification.

Recombinant PD-L1 bead binding assay (in-channel)

We imported PD-L1 coated beads, in suspension with a fluorescently labeled anti-mouse secondary antibody (AF568), into the main channel of the OptoSelect 11k chip so that beads were concentrated around the mouth of each NanoPen chamber. Secreted antibodies diffused from the NanoPen chambers into the channel where binding of the secreted antibody was detected optically as in-channel “blooms” in the TRED imaging channel. Blooms observed over the center of the NanoPen indicated positive PD-L1 binding.

Cell binding assay (in-pen)

The in-pen cell binding assay was performed by first co-incubating plasma B cells and CHO-K1-PD-L1 cells for 1 hour to allow for secreted antibodies to saturate the receptors. A fluorescently labeled anti-mouse secondary antibody (AF568) was then perfused through the OptoSelect 11k chip and allowed to diffuse into the NanoPen chambers. Anti-PD-L1 cell-binding antibodies were identified by locating pens with fluorescent CHO-K1-PD-L1 cells when imaged on the Beacon system using a TRED filter cube.

Ligand/receptor-blocking assay (in-pen)

After completing the in-pen cell binding assay, we perfused a fluorescently labeled, soluble PD-1-Fc fusion protein (AF488) through the OptoSelect 11k chip. PD-1 binding to the reporter cells was detected in the FITC imaging channel. NanoPen chambers containing CHO-K1-PD-L1 cells that are positive in both the TRED and FITC channels confirm the presence of secreted antibodies that have PD-L1 binding, but no blocking activity. NanoPen chambers that contained CHO-K1-PD-L1 cells that were positive in TRED but negative in FITC contained secreted antibodies that had both PD-L1 binding and PD-1/PD-L1 blocking activity.

Sequence recovery and functional confirmation

We exported cells secreting PD-L1/PD-1 blocking antibodies from specific NanoPen chambers to a 96-well PCR plate. Antibody heavy and light chain sequences were amplified and recovered using components of the Opto™ Plasma B Discovery cDNA Synthesis Kit and the Opto™ Plasma B Discovery Sanger Prep Kit, Mouse (Bruker Cellular Analysis). Recovered sequences were cloned into expression constructs, and antibodies were re-expressed and purified. Antigen binding and blocking activity was confirmed using plate-based ELISA and FACS measurements.

RESULTS AND DISCUSSION

Identifying blocking antibodies using a ligand/receptor blocking assay

An in-channel recombinant PD-L1 bead binding assay (Figure 3A, orange box) and an in-pen cell binding assay (Figure 3B, blue box) were first performed simultaneously to identify antibodies that bound recombinant PD-L1 and native PD-L1 expressed on the cell surface of a reporter cell, respectively.

Following the recombinant and cell-based binding assays, a PD-1/PD-L1 ligand/receptor-blocking assay was performed in-pen (Figure 3C). Fluorescent imaging clearly revealed antibodies that effectively blocked the ability of the fluorescently labeled PD-1 to bind PD-L1 expressed on CHO-K1 cells (Figure 3C, middle panel) as well as antibodies that were not effective blockers (Figure 3C, right panel) despite binding to PD-L1 in the recombinant and cell-based binding assays (Figure 3 A and B).

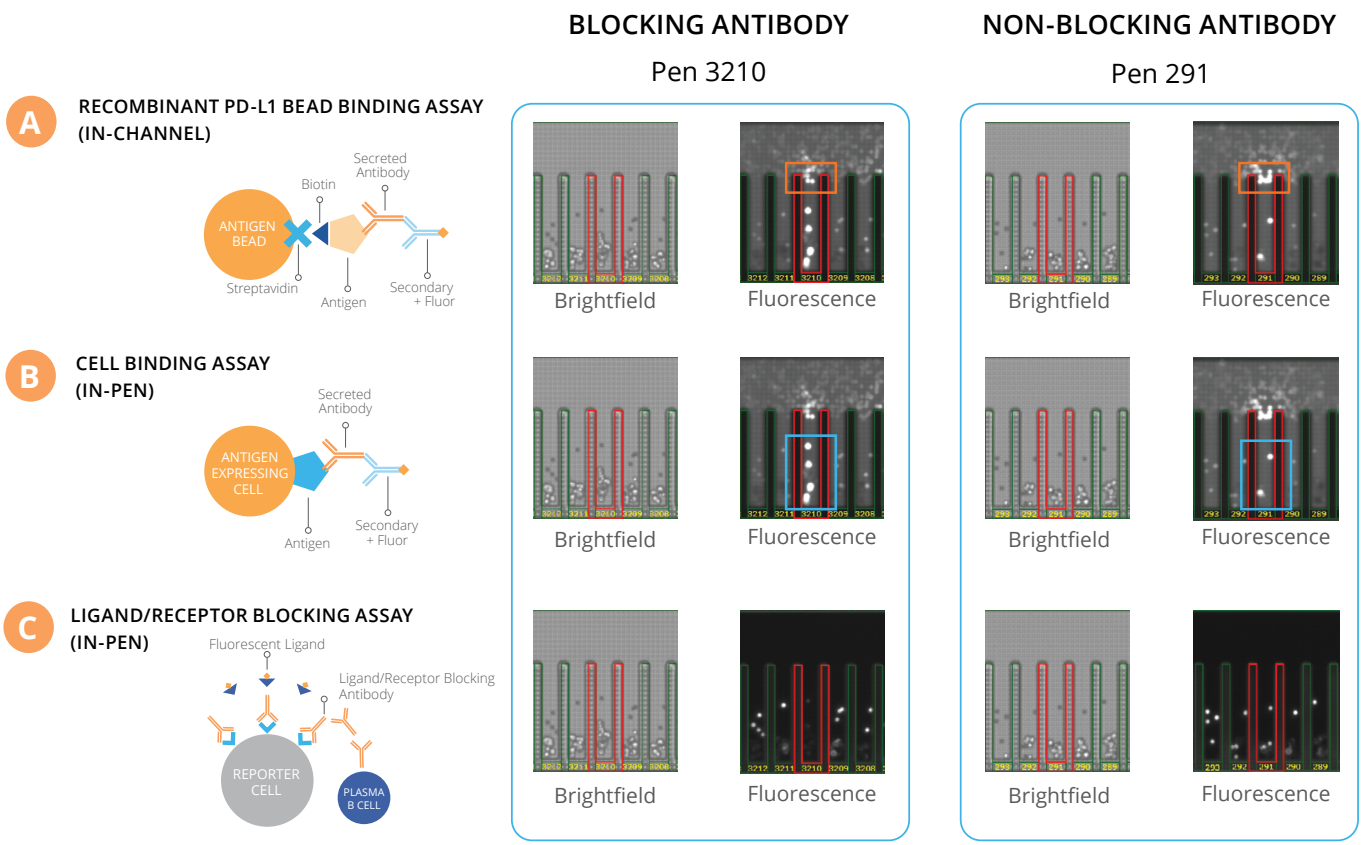


Figure 3. The Beacon system can perform multiple assays, simultaneously or in parallel, in just one day. (A) The recombinant PD-L1 bead binding assay performed in-channel (**orange** box) down-selects for antibodies that bind to the PD-L1 coated beads. In the examples shown, both the blocking and non-blocking antibodies bind the PD-L1 beads. **(B)** The cell binding assay performed in-pen (**blue** box) was performed at the same time as the recombinant assay and identifies antibodies that bind to native PD-L1 expressed by the reporter cell. In the examples shown, both the blocking and non-blocking antibodies bound the reporter cell. **(C)** The ligand/receptor-blocking assay identifies antibodies with the ability to block the PD-1/PD-L1 interaction. In the examples shown, the blocking antibodies are detected by non-fluorescent reporter cells, while the non-blocking antibodies result in fluorescent reporter cells.

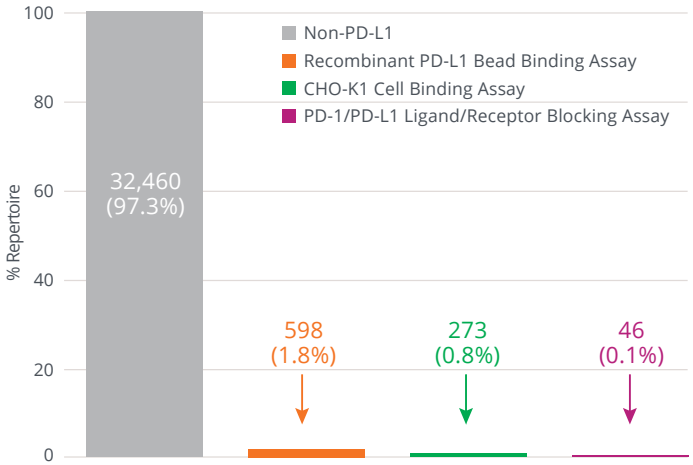


Figure 4. Deeper characterization enables down-selection of high-quality lead candidates. Fewer than 2% of screened plasma B cells secreted antibodies that bound recombinant PD-L1 (**orange**). Of these 598 antibodies, only 273 antibodies (fewer than 1% of plasma B cells screened) bound to the cell-based PD-L1 (**green**). Further screening with the ligand/receptor-blocking assay down-selected 46 lead candidates (0.1% of plasma B cells screened, **red**).

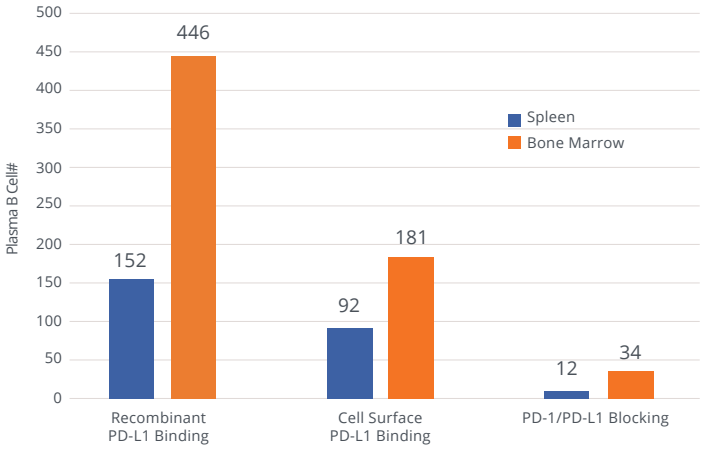


Figure 5. Opto Plasma B Discovery can identify a large number of functionally-active lead candidates by screening B cells from multiple organs. 3x more ligand/receptor blocking antibodies identified from plasma B cells in the bone marrow compared to the spleen (34 of the 46 candidates, or 74%).

Of the 33,377 mouse plasma B cells screened (16,500 cells from the spleen and 16,877 cells from the bone marrow), 598 (1.8%) cells generated antibodies that bound to the PD-L1 coupled beads. The cell binding assay allowed us to down-select further to 273 (0.8%) cells that secreted antibodies that bound to PD-L1 expressed on the surface of the CHO-K1 cells (Figure 4). The ligand/receptor-blocking assay identified 46 (0.1%) lead candidates that both bound PD-L1 and were able to block the interaction between fluorescently labeled PD-1 and PD-L1. The ability to down-select to 46 lead candidates eliminated the need to sequence, clone, re-express, and purify nearly 600 antibodies.

Discovering more blocking antibodies by accessing bone marrow plasma B cells

Opto Plasma B Discovery is uniquely capable of accessing plasma B cells from multiple organs, including spleen, bone marrow, and lymph nodes. We identified 3x more blocking antibodies by screening plasma B cells from bone marrow compared to the spleen plasma B cells (Figure 5), suggesting that this B cell compartment could be an important source for therapeutic molecules.

The plasma B cells secreting PD-1/PD-L1 blocking antibodies were unloaded from the chip for cDNA recovery and amplification of antibody heavy/light chain genes for sequencing. After sequencing the PD-1/PD-L1 blocking antibodies, we confirmed that the lead

candidates we identified using the Beacon instrument were unique antibodies compared to commercially-approved antibodies currently in the clinic (Figure 6).

Identifying antibodies with performance comparable to commercially-approved antibodies

We selected 24 blocking antibodies to clone, re-express and purify for characterization using orthogonal assays (Figure 7). 20 out of 24 antibodies (83%) of antibodies bound the extracellular domain (ECD) of human PD-L1 as confirmed by ELISA (Figure 7A). This binding was not limited to just recombinant proteins, as 20 of 24 antibodies also bound to CHO-K1 cells expressing the PD-L1 protein (Figure 7B). We also determined that these candidates bound the cynomolgus PD-L1 variant (Figure 7C), an important requirement for pre-clinical animal toxicological studies. Finally, we confirmed the lead candidates had functional ligand/receptor blocking activity in wellplate-based assays (Figure 7D). Of the 20 antibodies tested, 5 had IC50 values comparable to commercially-available therapeutic antibodies and 2 had sub-nanomolar affinities based on results generated using a Biacore instrument (GE Healthcare, data not shown).

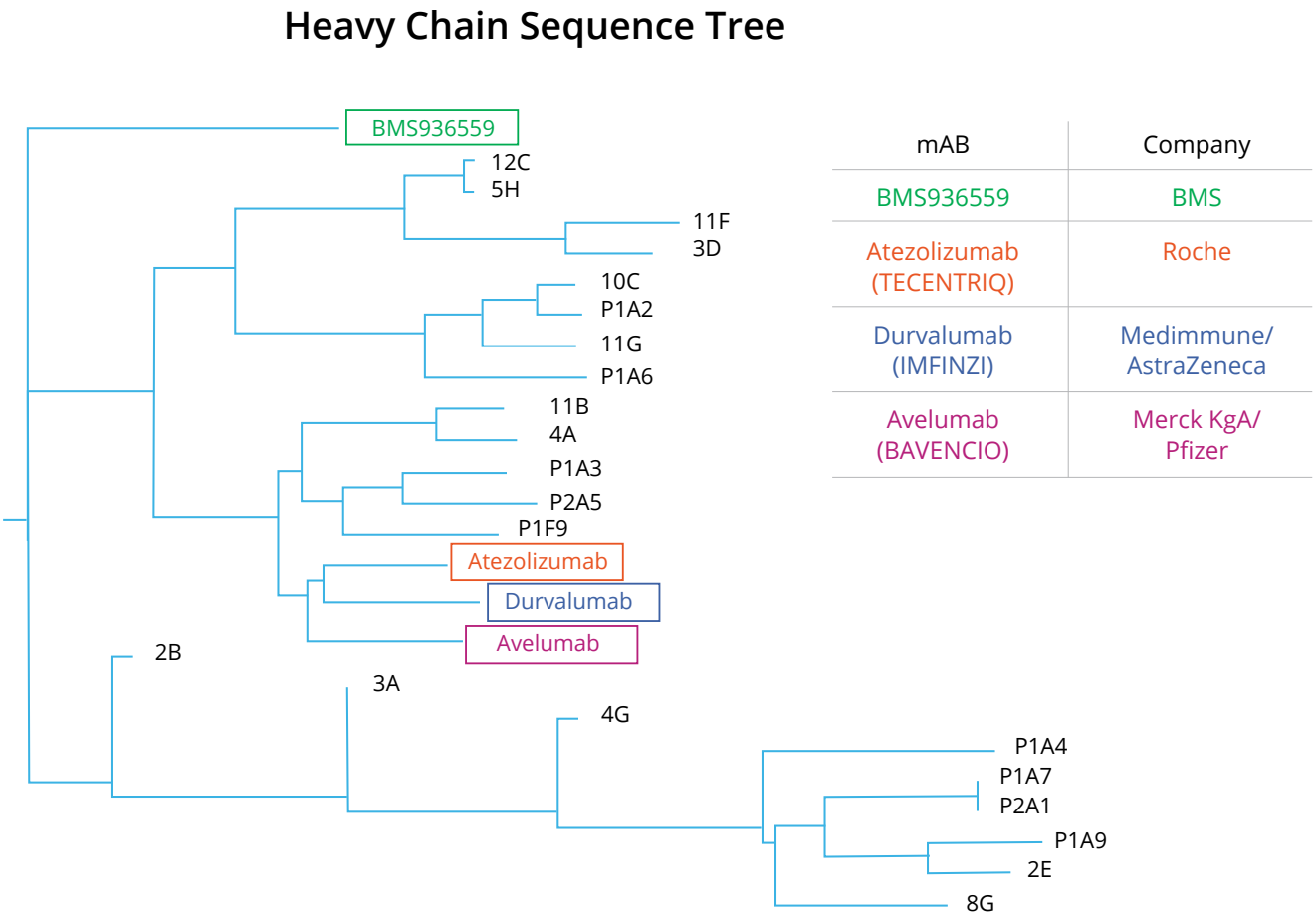
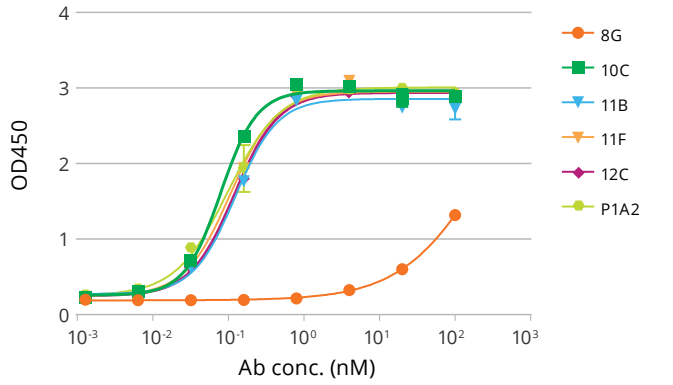


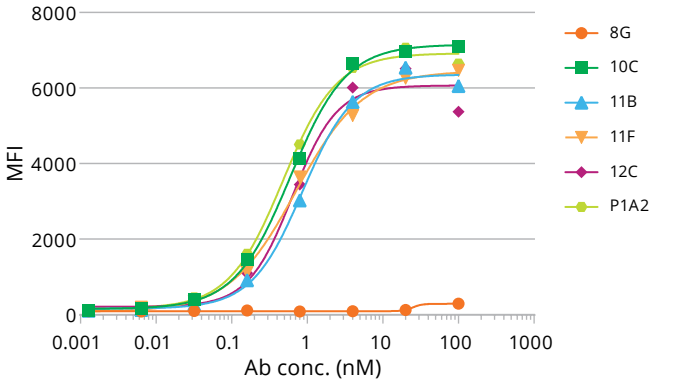
Figure 6. Opto Plasma B Discovery identifies unique antibodies. Comparison of the CDR3 sequences revealed unique ligand/receptor blocking antibodies distinct from previously commercialized antibodies. Data generated in collaboration with Shanghai ChemPartner Co., Ltd.

A 20 bound the PD-L1 ECD Fragment (ELISA)



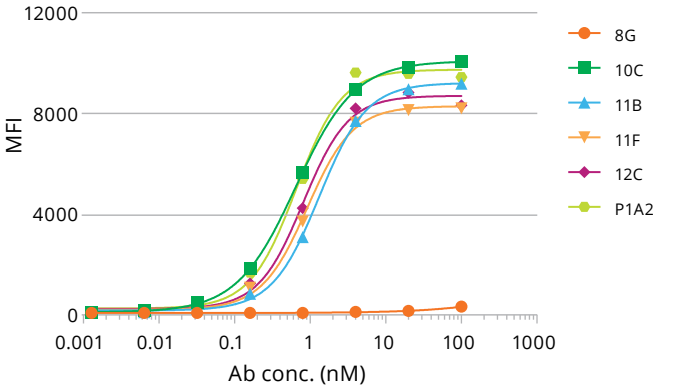
	8G	10C	11B	11F	12C	P1A2
EC50	228.4	0.07928	0.1239	0.1069	0.1215	0.09893

B 20 bound the PD-L1 expressed on CHO-K1 cells



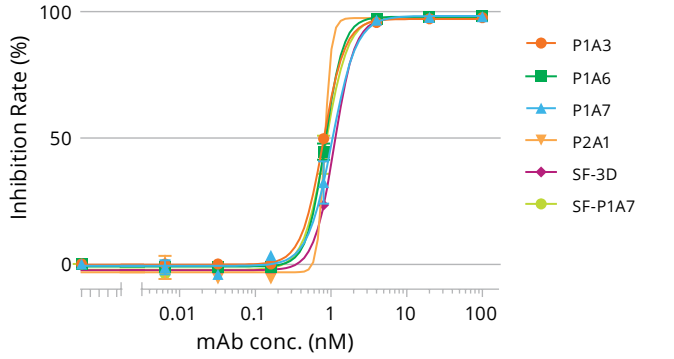
	8G	10C	11B	11F	12C	P1A2
EC50	~ 24.87	0.5987	0.8652	0.7082	0.6379	0.4760

C 20 bound the cynomolgus PD-L1 species variant



	8G	10C	11B	11F	12C	P1A2
EC50	~ 1.123e+007	0.6489	1.319	0.9160	0.8130	0.6537

D 20 bound and blocked PD-1/PD-L1 interaction



	P1A3	P1A6	P1A7	P2A1	SF-3D	SF-P1A7
IC50	0.7869	0.8366	1.032	~0.8212	1.112	0.8506

Figure 7. Re-expressed antibodies exhibited the expected functional behavior when evaluated using conventional techniques. 20 of 24 of the lead candidates that were cloned and purified exhibited binding affinity to the PD-L1 ECD using ELISA **(A)** and to the full-length protein expressed by CHO-K1 by FACS **(B)** The same 20 antibodies also bound to the cynomolgus PD-L1 that would most likely be used in required animal studies during the pre-clinical phase of drug development **(C)**. Finally, 20 of the purified antibodies effectively blocked the PD-1/PD-L1 interaction **(D)**. 20% of these antibodies had IC50 values comparable to PD-1/PD-L1 blocking antibodies currently in the clinic. Data generated in collaboration with Shanghai ChemPartner Co., Ltd.

CONCLUSION

Identifying well-characterized, functionally-relevant antibody lead candidates when screening plasma B cells early will reduce costly and time-consuming cloning and testing of non-functional, irrelevant candidates during the antibody drug development process. The Opto Plasma B Discovery workflow from Bruker Cellular Analysis not only makes it possible to screen the plasma B cell repertoire with multiple assays for better candidate characterization, but it does so in a single day, saving scientists crucial time. It also screens the B cell repertoire from multiple organs such as spleen and bone marrow to generate a greater diversity of lead candidates.

Using the PD-1/PD-L1 interaction as a model system, we demonstrated that multiple binding and blocking assays enabled the selection of 46 unique lead candidates capable of blocking the interaction between PD-1 and PD-L1. We also showed that 3x as many antibodies can be identified using bone marrow B cells (34 antibodies) compared to B cells from the spleen (12 antibodies), expanding the diversity of lead candidates.

The ability to perform a comprehensive screen on the B cell repertoire using multiple assays to identify functionally-relevant lead candidates against your target of interest – in just one day – may reduce the time and effort to bring new, efficacious therapies to the clinic.

For more information, visit

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