ChemPartner
Dedicated to LifeScience

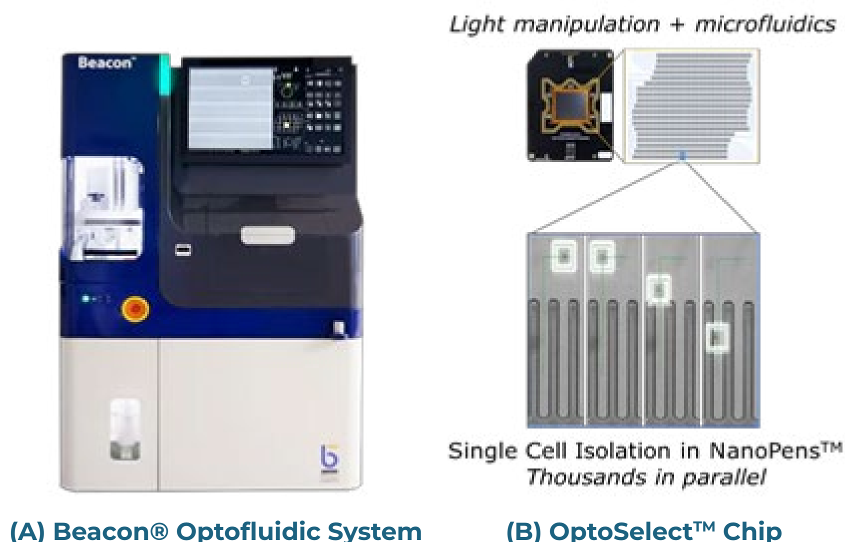
A CASE STUDY:

Accelerated antibody discovery of high quality anti-PDL1 antibodies using the Beacon® system

Monoclonal antibodies (mAbs) against PDL-1 were rapidly developed by the ChemPartner Biologics group using Berkeley Lights' Beacon® optofluidic single B-cell cloning technology. The high-throughput automation of the Beacon® system enabled us to identify PD-L1 blocking antibodies within a single day, significantly accelerating a process that would normally take 2–3 months using conventional methodologies such as hybridoma technology or synthetic display library. Using the Beacon®, we screened tens of thousands of primary single plasma B cells to identify unique antibodies against PD-L1. A series of assays were used for direct screening on the Beacon®. These assays included bead-based binding/blocking assays, cell-based binding to CHOK1 cells overexpressing human PD-L1, and cell-based blocking assays using PD-1. Once positive hits were identified, single plasma B cells were exported by the Beacon for antibody sequence recovery. After single-cell reverse transcription, amplification, and sequencing by NGS, the sequence diversity of the anti-PD-L1 antibody hits was evaluated. In addition, this technology can enable rapid comparison of target-specific repertoire diversity between different immunization strategies, mouse strains, and lymphoid organs. Through the rapid generation of diverse, high-affinity mAbs, clients can take advantage of this emerging technology to maximize their research success and advancement toward their commercial goals.

Figure 1. Introduction to single B-cell cloning technology for antibody discovery

(A) The Beacon® blends novel nanofluidic design with semiconductor technology to enable thousands of single cell experiments in parallel. (B) BLI's OptoSelect chip overlays a perfusion-based nanofluidic system over light-activated phototransistor arrays. This enables the rapid and precise positioning of single cells or other micro-objects using controlled patterns of light. The OptoSelect chip contains thousands of flow-isolated nanoliter chambers, called NanoPens, which physically isolate cells from flow but enable diffusion-based exchange.



(A) Beacon® Optofluidic System (B) OptoSelect™ Chip

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Figure 2. Beacon® plasma B-cell workflow for quickly assessing desired binding, blocking or other functional properties

(A) The conventional hybridoma workflow starts with animal immunizations, then proceeds with tissue harvest of lymphoid cells followed by fusion of the lymphoid cells with myeloma cells. After one or more rounds of sub-cloning and repeated screening assays, antibodies are scaled up for purification and further characterization. The post-fusion but prior to scale-up process typically takes 8-12 weeks. (B) The Beacon® condenses the 8-12 week workflow into a single day by enabling direct evaluation of plasma B cells secreting target-specific functional antibodies. Antibodies can be assayed for desired binding and blocking or other functional properties directly on the Beacon.

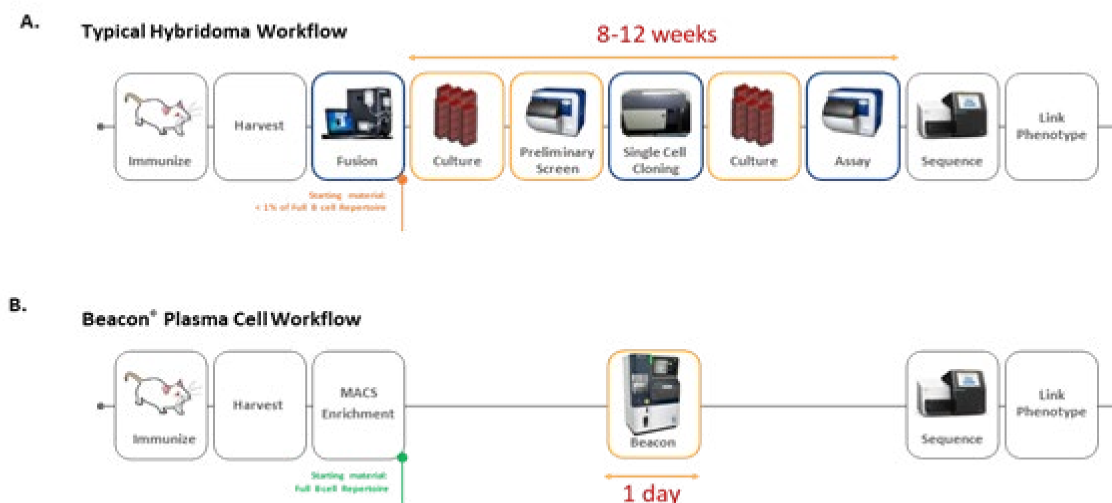


Figure 3. Plasma B-cell cloning workflow for antibody generation on the Beacon® system

Immunizations were performed using recombinant human PD-L1 ECD. After 10 weeks, bone marrow and spleen were harvested and isolations were done within 1-3 hours after harvest using the Miltenyi CD138 plasma B cell isolation kit. Isolated plasma B cells were loaded onto Beacon and binding experiments were done on huPD-L1 expressing CHOK1 cells and on beads coated with huPD-L1. Subsequently, blocking experiments were done on both cells and beads using soluble PD-1 conjugated to Alexa Fluor-488 (AF488). Once positive binders and blockers were identified, single plasma B cells were individually exported into a 96 well plate for further processing and single cell sequencing.

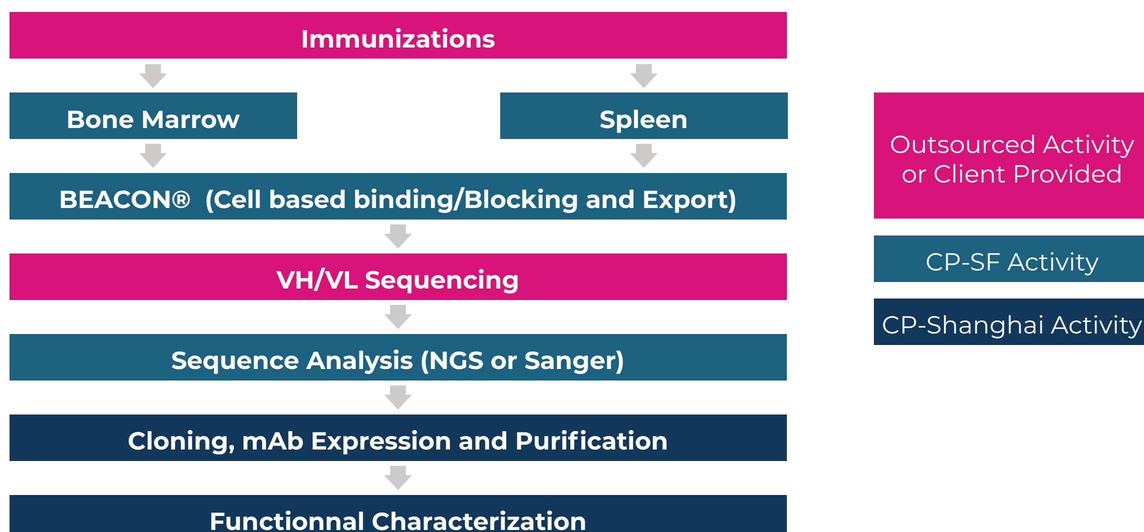


Figure 4. Flow cytometry analysis and characterization of enriched plasma B cells

Cells were isolated from spleen and bone marrow with CD138 isolation kit. Samples were taken pre- and post-isolation and stained with anti-CD138 PE, anti-CD3 BV605, anti- IgM AF488, anti-IgG1 APC antibodies, and Zombie NIR. (A) A majority (~80%) of the cells from spleen were CD138+ with up to 7% CD3+ cells present, suggesting that T cells are a slightly contaminating population from this organ. In contrast, the bone marrow plasma B cell preparation showed no CD3 staining with ~25% of the population being CD138+. (B) Cells were gated on CD138+ and CD3- staining prior to analysis of IgM and IgG staining profiles. Whereas IgM staining was evident prior to plasma B cell isolation, the CD138+ sub-population had minimal cells expressing IgM post-isolation in both the bone marrow and spleen preparations.

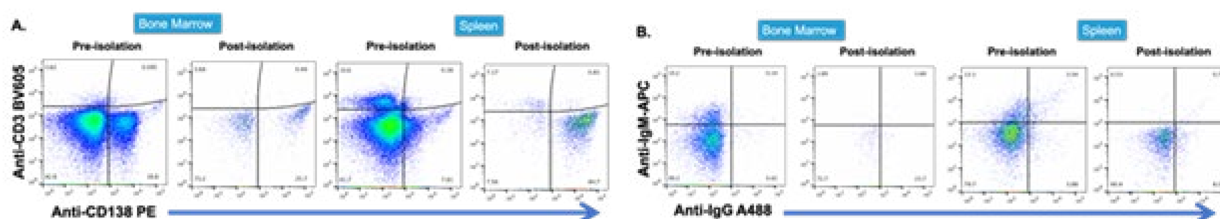


Figure 5. Representative cell- and bead-based binding and assays on the Beacon® System

Representative images from the Beacon® for in-channel/in-pen cell- and bead-based binding and blocking assays are shown. Target-specific secreted antibodies are visualized with anti-mouse IgG AF568 on both cells and beads in-pen and in-channel for the binding assays. This enabled identification of NanoPens containing single plasma B cells secreting target specific binding and potentially blocking antibodies. Once the beads and secondary antibodies were washed away, human PD-1 AF488 was imported which enabled scoring of cell- and bead-based blocking signals as shown. If AF488 signals were weak in the presence of antibodies, these antibodies were considered to have ligand-blocking properties. If AF488 signals were strong along with strong cell- or bead-based binding, these were considered binders that did not have blocking activity.

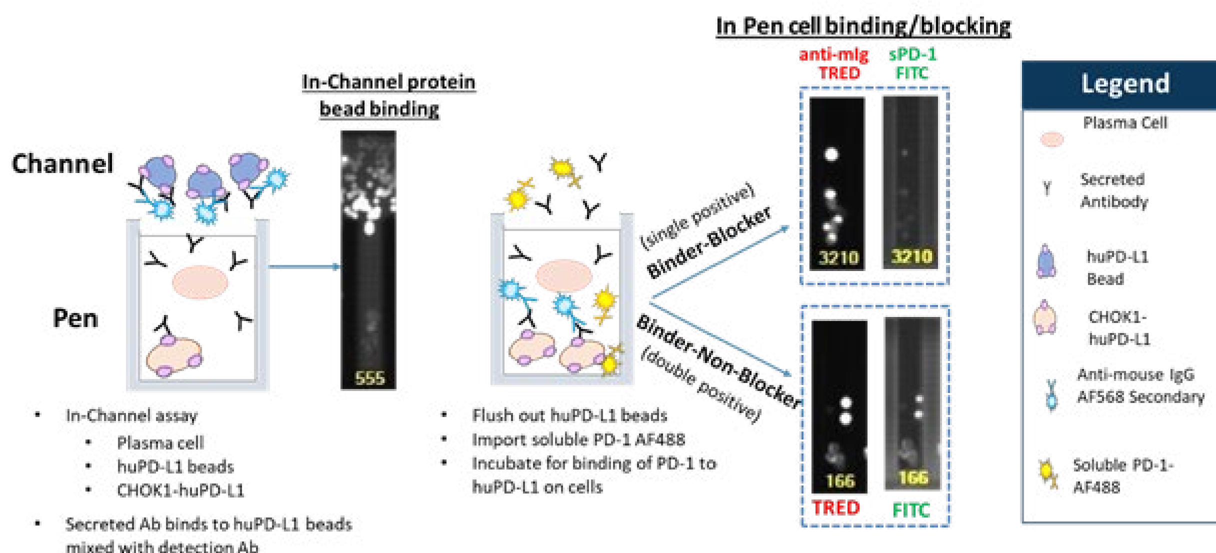
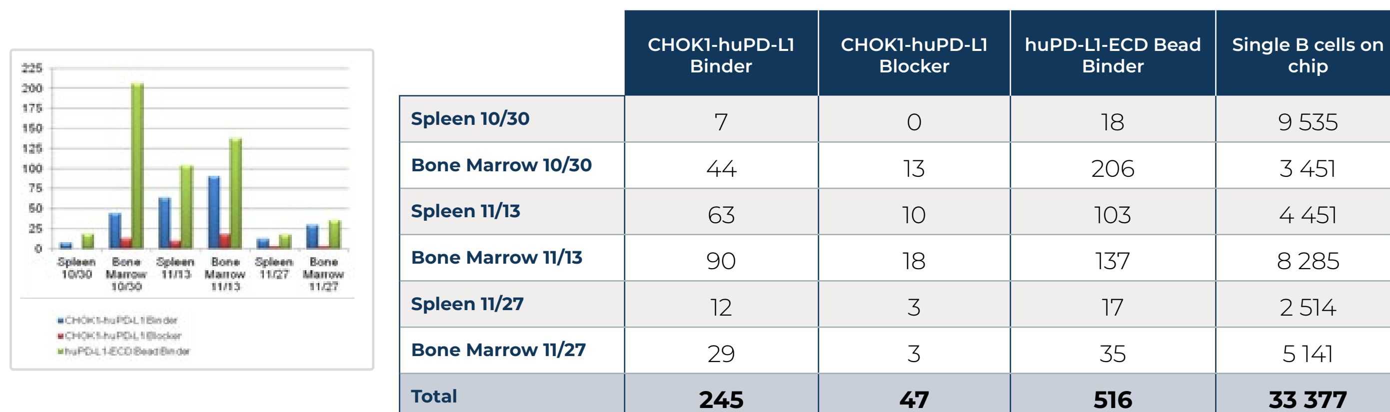


Figure 6. PDL-1 binders and blockers identified by Beacon® system

Data from three independent Beacon experiments are shown. Almost 13,000 single plasma B cells were evaluated in each of the first two experiments and over 7,600 cells were analyzed in the third experiment. In the first experiment (10/30 in the figures below), significantly more cell- and bead-based binders were identified from the bone marrow compared to the spleen of the same immunized mice. In the second study (11/13), more binders were identified from both the bone marrow and spleen, with 18 ligand-blocking antibodies identified from the bone marrow and 10 from the spleen. In the third study (11/27), far fewer total binders were identified than the first two studies, however three blocking antibodies were identified each from the spleen and bone marrow. After evaluating over 33,000 cells from the three combined studies, a total of 47 cells were identified that secreted antibodies with cell-based ligand-blocking properties. Of the 47 antibodies with blocking properties, 34 were isolated from bone marrow and 13 were isolated from spleens of immunized BALB/c mice.



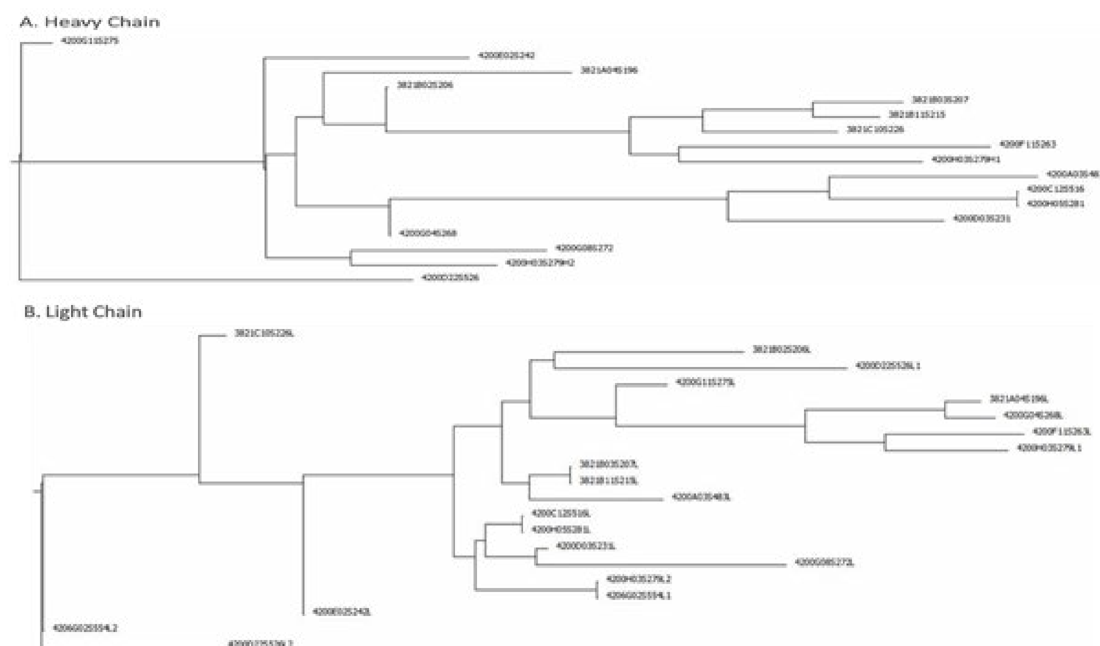
	CHOK1-huPD-L1 Binder	CHOK1-huPD-L1 Blocker	huPD-L1-ECD Bead Binder	Single B cells on chip
Spleen 10/30	7	0	18	9 535
Bone Marrow 10/30	44	13	206	3 451
Spleen 11/13	63	10	103	4 451
Bone Marrow 11/13	90	18	137	8 285
Spleen 11/27	12	3	17	2 514
Bone Marrow 11/27	29	3	35	5 141
Total	**245**	**47**	**516**	**33 377**

Figure 7. Hierarchical clustering tree of heavy and light chain CDR3 sequences of antibodies identified on Beacon®

Plasma B cells were exported from the Beacon and paired heavy chain and light chain (VH/VL) sequences were recovered. Sequencing was done using a MiSeq (Illumina, Inc., San Diego, CA) and paired VH/VL CDR3 sequences were recovered using BLI's custom bioinformatics methods. Sequences were aligned to generate a hierarchical clustering tree for both heavy and light chain CDR3 regions.

(A) After comparing CDR3 sequences of 17 heavy chains, 16 were found to be unique.

(B) After alignment of CDR3 sequences from 20 light chains, 17 were identified as unique.



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Conclusions

The Beacon system allowed identification of target-specific binding and blocking antibodies in a fraction of the time it takes using standard hybridoma techniques. Binding activity was assessed on both cells and beads, followed by assessment of ligand-blocking activity on both. From three independent experiments, a total of 47 anti-huPD-L1 blocking antibodies were successfully identified and a majority were successfully exported. The Beacon platform enabled rapid discovery of target specific antibodies and interrogation of the immune repertoire across a variety of parameters, including lymphoid organs, immunization strategies, immunogens, and mouse strains, among others.

Taken together, the Beacon system accelerated the discovery of anti-huPD-L1 functional antibodies with desired properties. Importantly, by moving function forward, subsequent downstream activities, such as sequencing, cloning, purification and characterization can focus on clones with desired functional properties. This will allow researchers to improve the direction and the efficiency of the antibody discovery process.

For more information: https://www.chempartner.com/biologics/b-cell-cloning/

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