ChemPartner

Dedicated to LifeScience

Functional Evaluation of Unique Anti-PDL1 Antibodies Generated through Single Plasma B Cell Cloning on the Beacon® Platform Versus a Standard Hybridoma Approach

Nitin Patel[1], Neha Yevalekar[1], Ye Jin[1], Dandan Lv[2], Yuehua Chen[2], Lina Xu[2], Zhenhui Xie[2], Yingying Hu[2], Xinyi Guo[2], Mingming Pan[2], Xiaoni Zhan[2], Rui Wang[2], Vincent Pai[3], Minha Park[3], Ravi Ramenani[3], Maryam Shansab[3], Po-Yuan Tung[3], Amanda Goodsell[3], Adrienne Higa[3], Daniel Bedinger[4], Teddy Yang[2], Shireen Khan[1]

[1]ChemPartner San Francisco, South San Francisco, CA; [2]Shanghai ChemPartner Co., Ltd., Shanghai, China; [3]Berkeley Lights, Inc., Emeryville, CA; [4]Carterra, Inc., Salt Lake City, UT.

BERKELEY LIGHTS

ABSTRACT

PD-L1 is a key inhibitor of T cell activation that is often over-expressed in cancer to escape immune surveillance and promote tumor progression. Blocking antibodies against PD-L1 or its receptor, PD-1, have shown significant clinical benefit in some patients with PD-L1 expressing tumors. Hence, there is great interest in generating therapeutic antibodies against these targets to counteract the immune suppression mechanism that tumors rely on for survival. Most of the anti-PD-L1 therapies in the clinic have been generated by standard hybridoma technology. We investigated whether superior anti-PD-L1 antibodies with greater diversity, affinity, and/or functional activity could be generated using single B cell cloning (BCC) which could circumvent a labor-intensive and time consuming process. Thus, we generated unique antibodies against PD-L1 via both methods. The Beacon® platform (Berkeley Lights, Inc., (BLI)) enabled screening of 33,377 antibody secreting plasma B cells (PCs) against PD-L1. After penning CD138$^+$ single PCs onto OptoSelect™ chips, we identified over 200 antibodies with binding to PD-L1, and of those, 35 antibodies blocked binding of PD-1 to PD-L1. The hits were exported for antibody sequence recovery from single B cells and 24 hu-IgG4 chimeric antibodies were generated. For the traditional hybridoma approach, Balb/c and SJL mice were immunized with recombinant PD-L1 and 13,536 hybridoma cells were screened. We purified 44 hybridoma antibodies and 24 BCC antibodies which were characterized for FACS binding, receptor blocking, epitope binning using the Carterra LSA and affinity by Biacore 8K and Carterra LSA. Image based selection in the blocking assay enabled identification of 58% true blockers by Beacon with IC50s comparable to benchmarks. We found that 2 antibodies from Beacon and 3 antibodies from hybridoma had triple-digit pM affinity. After assessing the epitope diversity, our data shows that hybridoma Abs covered more diverse epitope space than BCC. Taken together, our data show that plasma B cells secreting functional antibody candidates can be rapidly identified on Beacon compared to several months for a hybridoma campaign, thus substantially accelerating the antibody discovery process. Although the hybridoma approach allowed recovery of a greater number of blocking antibodies spanning a broader epitope space, B cell cloning on the Beacon platform enabled greater access to the immune repertoire coupled with the ability to screen through robust functional assays up front, thus enabling identification of high affinity, potent anti-PD-L1 antibodies.

MATERIALS AND METHODS

Immunizations: Balb/c mice for Beacon study or Balb/c and SJL mice for hybridoma study were immunized by i.p. injection with recombinant human PD-L1 ECD protein using CFA/IFA adjuvants at Pacific Biolabs (Hercules, CA) and ChemPartner, Shanghai, respectively. Titers were measured at day 21 and 42 by ELISA; spleen and bone marrow were harvested at day 57 for PCs isolations and electrofusion for Beacon and hybridoma approach, respectively.

PCs Isolations & Single B cell antibody screening assay for the Beacon: Single cell suspensions were prepared from bone marrow and spleen. Ficoll gradient performed to remove RBCs from spleen. PCs were isolated using the CD138$^+$ mouse plasma cell isolation kit and penned onto optoselect 3500 chip. Bead and cell based binding/blocking assays were performed simultaneously using SA beads coated with huPD-L1 and anti-mouse-IgG AF568, and CHOK1-huPD-L1 cells. To identify blocking antibodies, a soluble human PD-1 AF488 was imported to detect absence of ligand binding.

PCR Amplification/Sequencing/Cloning and Expression of Beacon hits (24): Paired HC and LC (VH/VL) sequences were amplified from single B cells using BLI's proprietary protocols followed by Sanger sequencing. The variable mouse domains for each antibody were cloned into a vector containing human IgG4 Fc (S228P), expressed in CHO cell line and purified (BCC-Abs).

Hybridoma screening funnel and purification of confirmed hits (44): Electrofusion generated hybridoma were screened using primary ELISA screen (144 plates/228 hits) and secondary cell-based hPDL1 binding screen (202 hits). The final receptor/ligand blocking screening identified 51 blockers, finally 44 were sub-cloned, reconfirmed their hPDL1 binding and purified (Hyb-Abs).

Determination of EC50 for cell binding: CHOK1-huPDL1 cells were treated with each antibody diluted serially 1:5 for a 7 point curve starting at 100nM. Following washing, the binding was detected by Alexa Fluor® 488 Goat Anti-human IgG (H+L) Antibody (1:1000 dilution). The cells were washed and resuspended in FACS buffer prior to FACS analysis.

Receptor/Ligand Blocking Assay: 96-well plates coated with 1μg/ml hPD-L1-ECD-Fc were incubated with antibodies (40nM, 1:2 serial dilution, 10 points), followed by adding 2μg/ml Bio-hPD1, incubated at 37 °C for 1 hr and washed 6 times with PBST. For detection, anti-streptavidin-HRP secondary antibodies (1:5000 dilution) were added, washed with PBST and developed using TMB substrate. OD values were recorded using Spectra max plus384 at 450nm.

Biacore: Anti-hFc antibody was immobilized on a Series S CM5 sensor chip. Kd measurements were estimated by capturing antibody (2μg/ml) and then flowing hPD-L1-his at a 2-fold titration series starting at 100nM. The flow rate was 30 μL/min with 180s association time and a 400s dissociation time. 10 mM glycine-HCl pH 1.5 was injected for 30s for regeneration.

Carterra LSA for binding kinetics and epitope binning: A covalent array was prepared using 23 BCC-Abs, 41 Hyb-Abs and 3 benchmark therapeutic abs (Tabs) at 10 and 0.5 μg/mL. Also, anti-His tag mA), huPD-L1 ECD His and PD-1 Ligand immobilized. On this array, brief kinetic measurements were generated using four serial concentrations of PD-L1 (4, 20, 100, 500 nM). The same array was then used for 67×67 classical sandwich epitope binning assay, where the antigen was injected over the array for 3 minutes, followed by an analyte antibody injection for 3 minutes. The surface was regenerated and this process was repeated for each antibody.

FIG 1. FLOW SCHEME OF IMMUNIZATION/ANTIBODY SCREENING

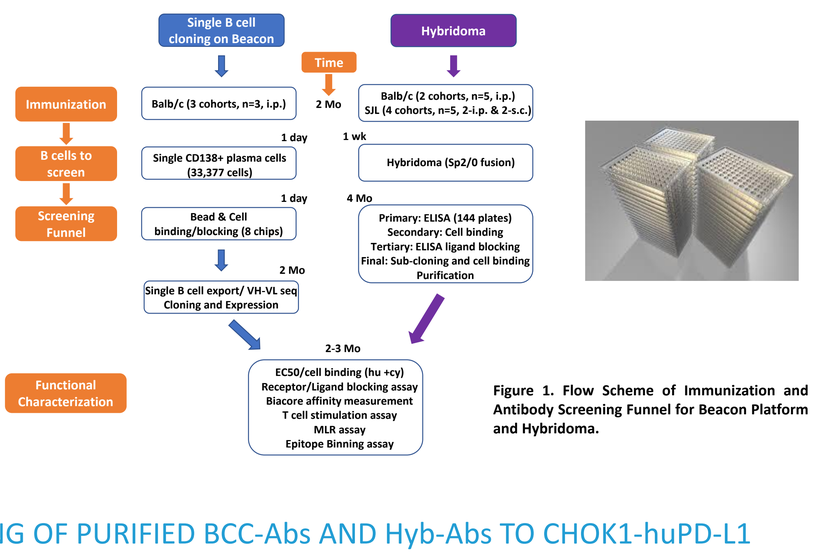

Figure 1. Flow Scheme of Immunization and Antibody Screening Funnel for Beacon Platform and Hybridoma.

FIG 2. FACS BINDING OF PURIFIED BCC-Abs AND Hyb-Abs TO CHOK1-huPD-L1

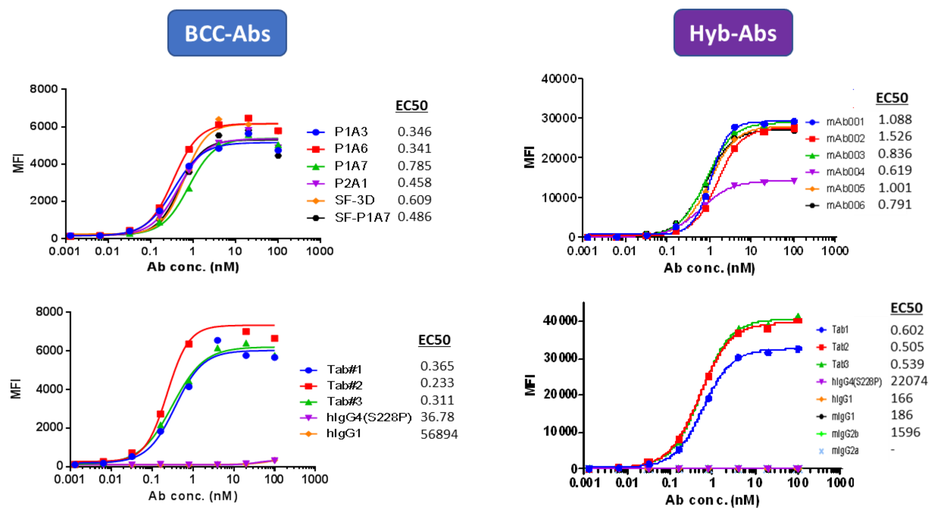

Figure 2. FACS Binding curve of Purified BCC-Abs (24) and Hyb-Abs (41) to CHOK1-huPD-L1. Total of 24 antibodies out of 35 blocking hits from Beacon were cloned as human IgG4 (S228P), expressed and purified. Purified IgGs were characterized for FACS binding at 100-0.001 nM concentration range. EC50 values were determined for each antibody. The data of 6 representative antibodies from BCC-Abs and Hyb-Abs, 3 Tabs and a panel of control antibodies is shown. 20/24 BCC-Abs and 37/41 Hyb-Abs bind to CHOK1-huPD-L1 cells; 5 BCC-Abs and 10 Hyb-Abs showed comparable EC50 values to Tabs.

FIG 3. RECEPTOR LIGAND BLOCKING ACTIVITY OF BCC-Abs AND Hyb-Abs

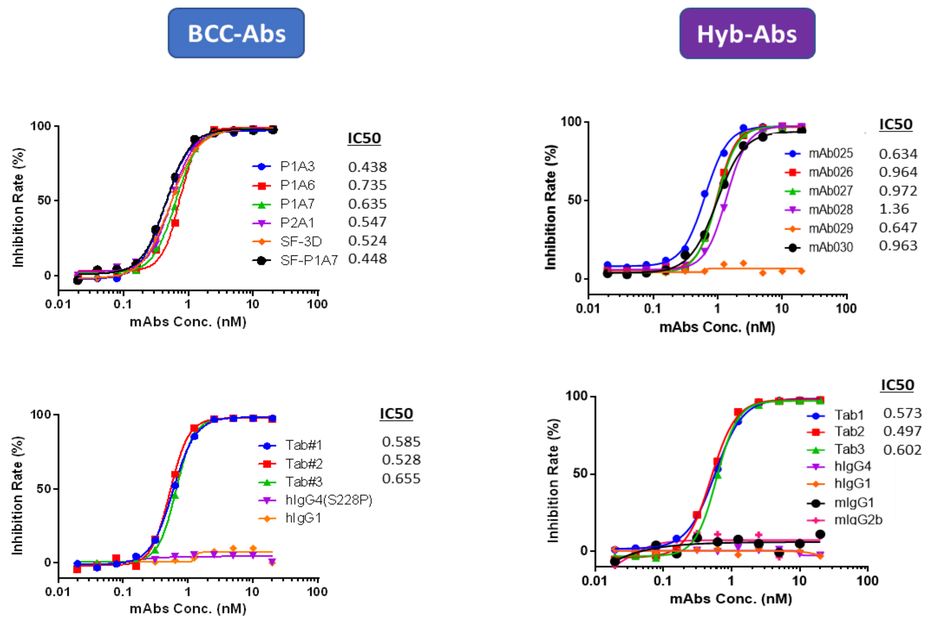

Figure 3. Receptor Ligand Blocking Activity of BCC-Abs (24) and Hyb-Abs (41). 19/24 BCC-Abs were ligand blockers with 5 Abs showing higher potency and 9 Abs with comparable potency to Tabs. 31/41 Hyb-Abs were ligand blockers with 11 Abs with comparable potency to Tabs.

FIG 4. BIACORE AFFINITY AND CARTERRA ISO-AFFINITY PLOT OF BCC-Abs, Hyb-Abs AND TABS

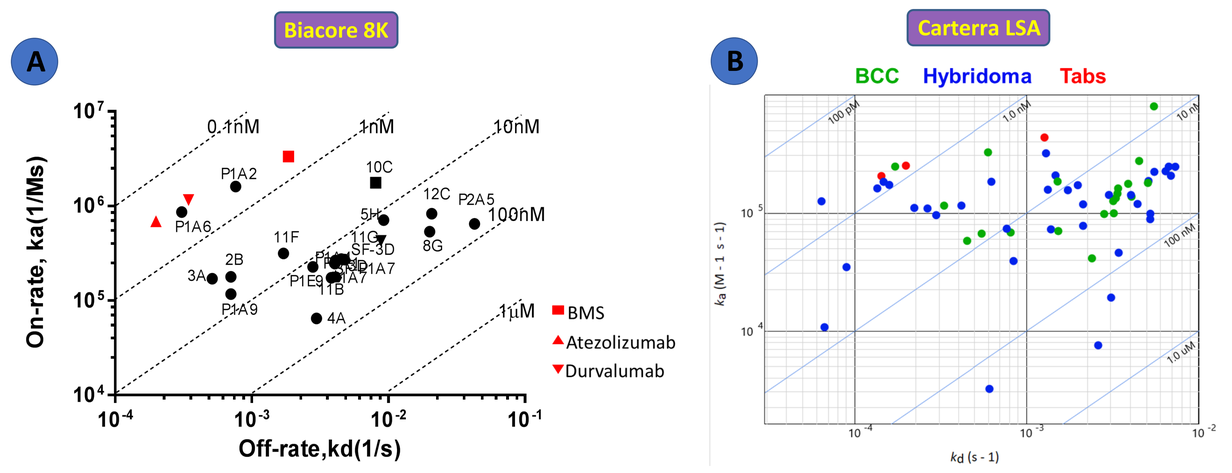

Figure 4. Biacore and Carterra LSA Affinity Analysis and Iso-Affinity Plot. (A) Iso-affinity plot was generated of BCC-Abs using Biacore data which showed a majority of clones had faster off-rates to human PD-L1 than Tabs. Two clones had comparable on and off rates relative to Tabs with sub-nM affinity, 5 clones had single digit nM and remainder had double digit nM affinities. **(B)** LSA iso-affinity plot was generated using kinetic measurements of four concentration points through LSA analysis software. Blue diagonal lines represent equivalent K_D.

FIG 5. CARTERRA HIGH THROUGHPUT EPITOPE BINNING OF BCC-Abs, Hyb-Abs AND TABS

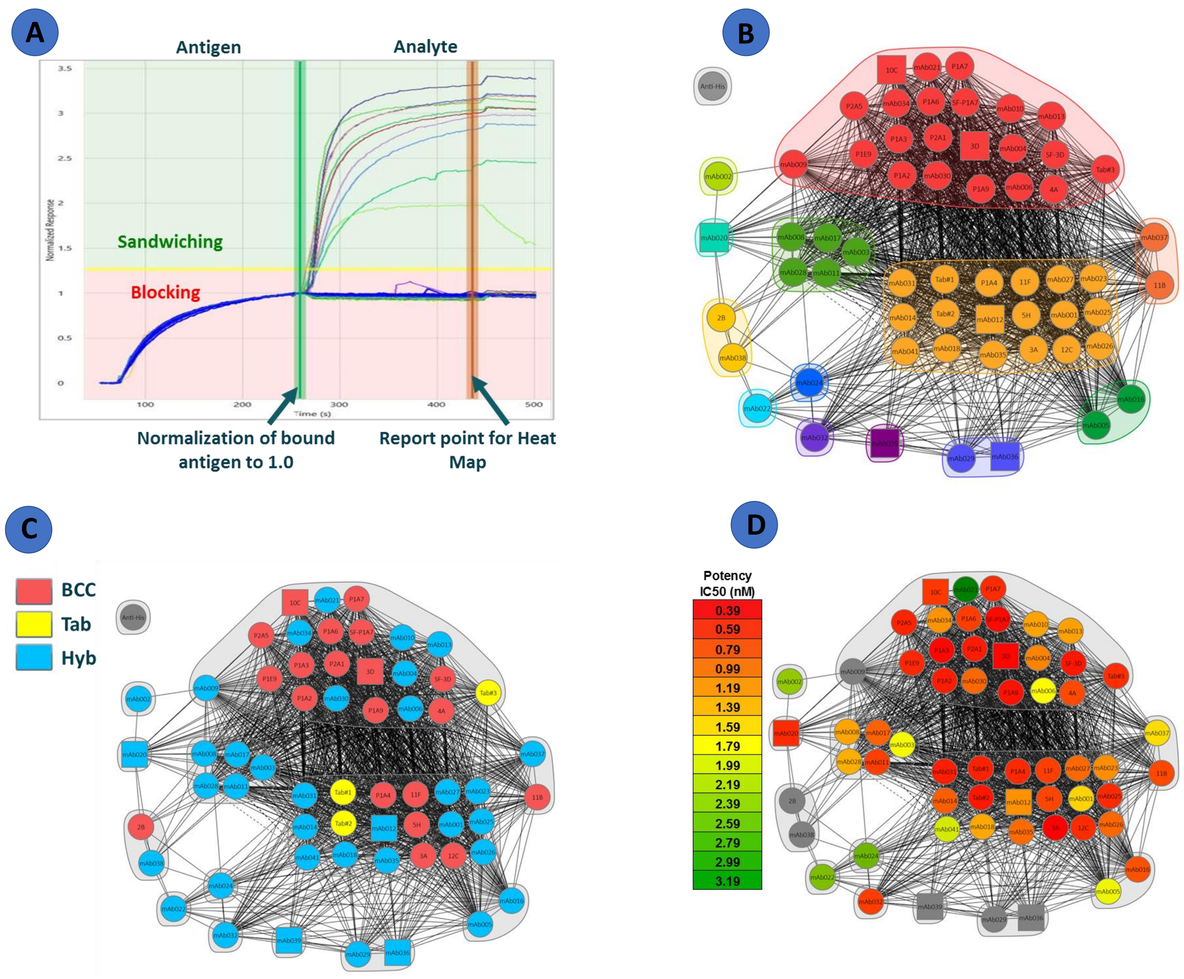

Figure 5. Carterra LSA Epitope Binning of BCC-Abs, Hyb-Abs and Tabs. A pair-wise classical epitope binning assay was performed on HC30M chip in a 67×67 array. The PD-L1 antigen was used at 50 nM and analyte antibodies used at 20 μg/mL. **(A)** Sensorgram data for P2A1 BCC-Ab. **(B)** Community plot showing a group of antibodies displaying highly related competition profile using cutoffs on the dendogram plot. Community plot colored by **(C)** source of antibody and **(D)** by their receptor/ligand inhibition IC50 potency.

FIG. 6 HIERARCHICAL CLUSTERING TREE OF PAIRED VH/VL SEQUENCES FOR BLOCKERS

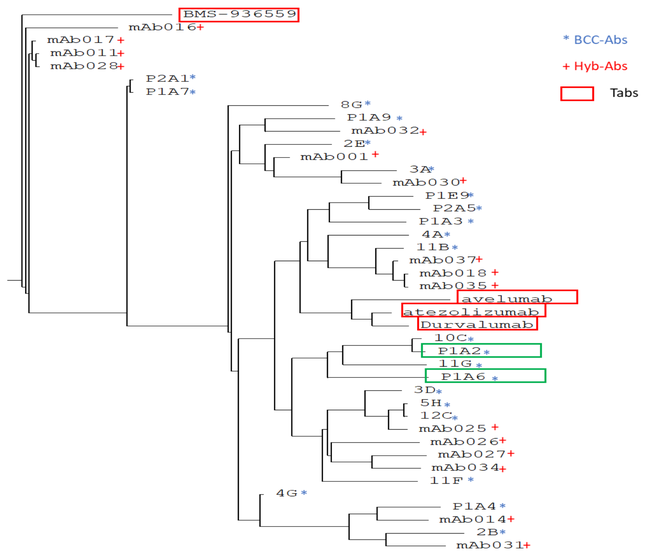

Figure 6. Hierarchical Clustering Tree of Paired Heavy and Light Chain Sequences of Blocking Antibodies Identified on Beacon® and Hybridoma. Single VH and VL sequences were recovered from B cells secreting blocking antibodies and hybridoma clones. Then, Sanger sequencing of the entire V region was completed on 22 BCC clones and 16 Hybridoma clones. V region sequences were aligned to generate a hierarchical clustering tree for paired heavy and light chains along with four benchmark anti-PD-L1 antibodies (Tabs). The two high affinity candidates with potent blocking activity from BCC cluster and are distinct from the Tabs (green boxes).

Functional Characterization Summary

	B Cell Cloning on Beacon	Hybridoma
Blockers in primary screen	35	>51
Single digit nM affinity	6	12
Blocking False Positive Rate	5/24 (21%)	13/41(32%)
Blocking IC50 ~ Tabs	14/24 (58%)	11/41 (32%)
Blocking IC50 < Tabs	5/24 (21%)	0/41
pM Affinity and potent blocking	2/2 (100%)	1/3 (33%)
Epitope Bins	4	13
T cell stimulation EC50 ≤ Tabs	9/24 (37.5%)	4/41 (9.7%)

CONCLUSIONS

The Beacon platform enabled rapid identification of unique, functional ligand blocking anti-huPD-L1 antibodies using three screening assays in a single day, while hybridoma involved multiple screening rounds across 2 months and required purification of antibodies to identify blocking clones.

BCC-Abs had a significantly lower false positive rate as compared to Hyb-Abs, thus a majority of BCC-Abs retained blocking activity as purified chimeras.

More importantly, five BCC-Abs showed higher blocking potency than Tabs, while none were identified for Hyb-Abs, lacking affinity-potency relationship.

LSA Epitope binning studies demonstrated that hybridoma Abs covered more diverse epitope space than BCC Abs. The plots showed striking correlations and predicted sequence similarities and diversity of the hybridoma and BCC antibodies as shown in the hierarchical clustering trees. Overall, the analysis revealed that the two most potent blockers from BCC with pM affinity are unique and not related to Tabs.

BCC offers greater sampling of the immune repertoire combined with robust image based functional interrogation, thus delivering antibodies with unique sequences, diversity and greater potency compared to the standard hybridoma method.

REFERENCES

1. Caraux A. et al., Haematologica 2010; 95(6): 1016-1020
2. Swaika A. et al., Molecular Immunology 2015; 67(2A): 4-17
3. Sivasubramaniana A. et al., MABS 2017; 9(1): 29–42

ACKNOWLEDGEMENTS

The authors wish to thank Louis Liu and Sarah Lively for support and thoughtful discussions.
We thank Christian Giddens and Tim Germann for their support with Carterra LSA analysis.