Powerdoma™: an upgraded hybridoma platform boasting fast timeline and improved cost-effectiveness for antibody discovery

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ABSTRACT

As one of the most traditional and well-established technologies, hybridoma approach contributed to more than three quarters of antibody drugs approved by FDA over the last decade. However, this technology is limited by several bottlenecks ever since its first appearance, including time-consuming process and loss of diversity in in cell fusion and subcloning. To address these limitations, here we developed the ***Powerdoma™*** platform, an upgraded version of the conventional hybridoma technology, which allows us to discover functional Ab leads with good diversity in a much faster and more cost-effective way.

BOTTLENECKS & SOLUTIONS

Diversity loss in conventional hybridoma workflow

Due to the nature of cell fusion and genomic instability of hybridoma cells, the diversity of Ab sequences carried by hybridoma cells suffers significant loss during each step of the screening process, leading to much fewer surviving candidates for functional screening and reduced chance of identifying functional Ab hits.

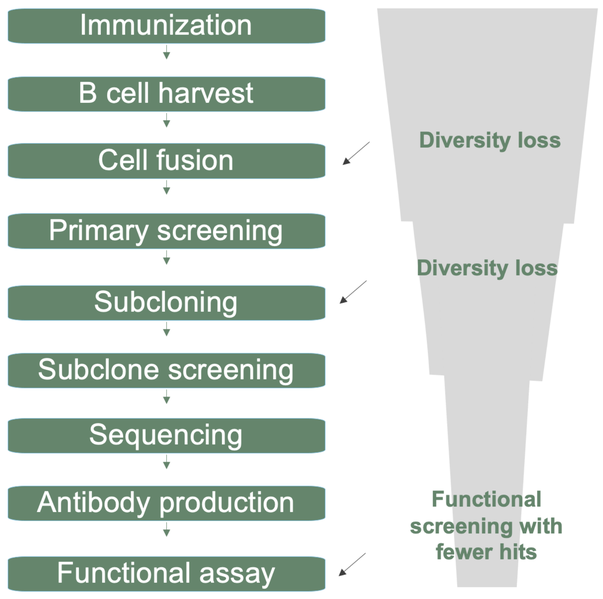

Figure 1

Powerdoma™ vs conventional hybridoma workflow

Conventional hybridoma (figure 2B) workflow heavily relies on the subcloning steps, which may take about 6 weeks, to reach the monoclonal stage of hybridoma clones for downstream screening. In contrast, this time-consuming subcloning process is eliminated in the Powerdoma workflow (figure 2A); instead, an optimized process is developed to allow the direct achievement of monoclonal stage of hybridoma clones. This optimization leads to a shortened timeline by 1-1.5 months and enables functional screening using hybridoma supernatant at much earlier stage of Ab lead discovery, allowing the identification of functional Ab hits with a much faster turnaround time, improved cost-effectiveness and better chance of success.

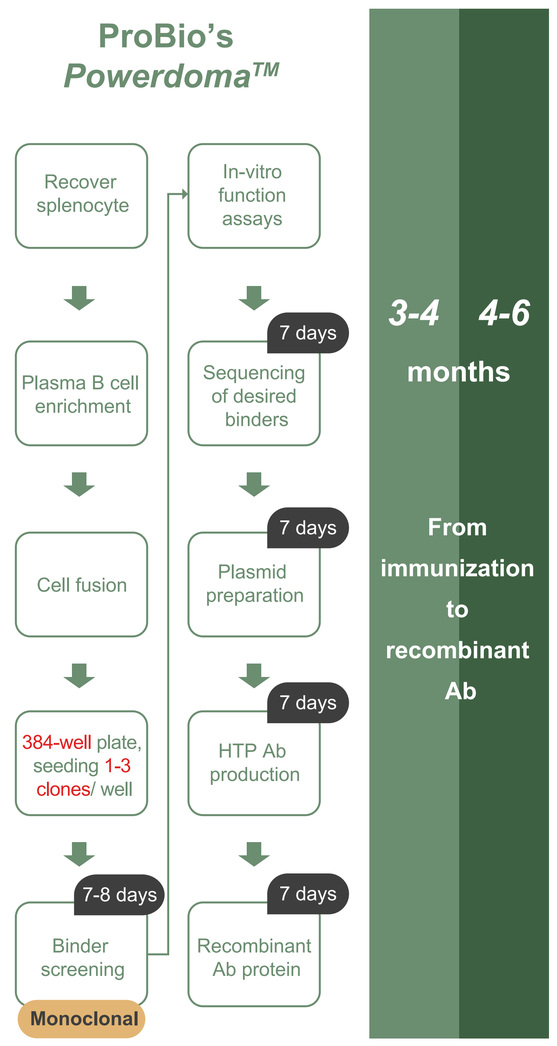

Figure 2A

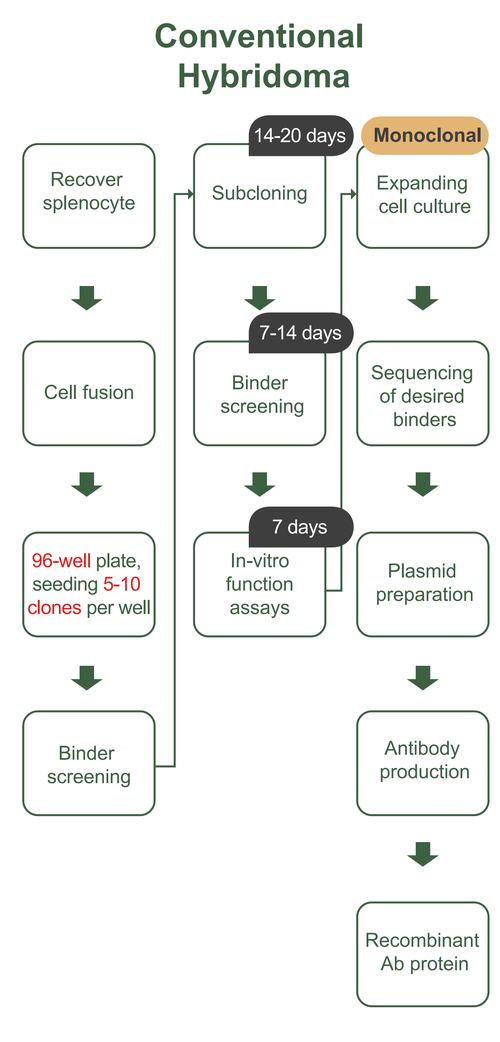

Figure 2B

Powerdoma™ project statistics

Comparing to conventional hybridoma workflow, ***Powerdoma™*** allows ~40% more hybridoma clones to be screened (figure 3A), with a shortened timeline decreasing from 58 to 30 days in average (figure 3B), and a four-fold higher positive hit rate (2000+ vs. 500+) in average from 11 projects with head-to-head comparison (figure 3C). Based on the 40+ previously delivered projects, for Powerdoma workflow we observed a monoclonal rate of 80%, a sequencing success rate of 97%, and a 100% confirmation rate by recombinant expression (figure 3D).

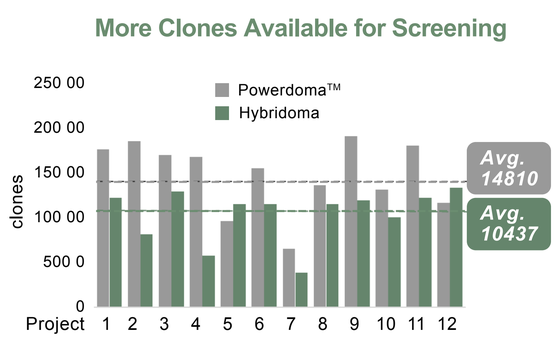

Figure 3A

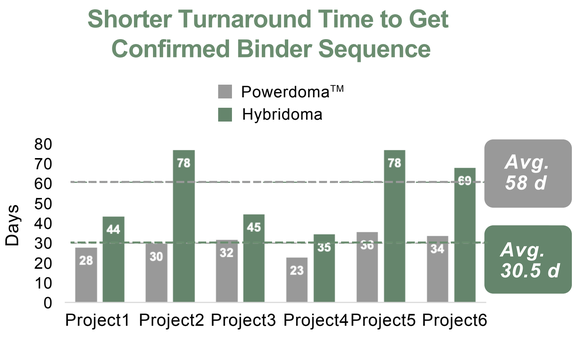

Figure 3B

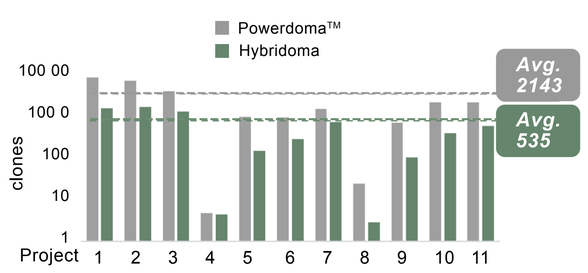

Figure 3C

40+
Powerdoma™ projects successfully completed

~80%
Monoclonal rate

>97%
Success rate of sequencing

100%
Recombinantly confirmed

Figure 3D

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FUNCTIONAL CHARACTERIZATION CASE STUDIES

1. Affinity ranking by KD-ELISA using *Powerdoma™* supernatant

Affinity ranking by SPR/BLI is expensive, especially when it comes to deal with hundreds of samples. As a cost-effective substitute, kD-ELISA utilizes a capturing Ab coated to the plate to capture the Abs being tested from the supernatant, followed by detection with a biotinylated antigen (figure 4A). In a primary screening assay using Powerdoma supernant based on kD-ELISA, a number of potentially strong binders (green box) and weak binders (red box) were identified (figure 4B). Then these selected candidates were recombinantly expressed and characterized by full curve ELISA. Consistently, the previously identified strong binders (clone 4, 7, 13, 15 in green box) showed a high binding affinity similar to benchmarks (figure 4C), while those weak binders (clone 1, 3, 14) showed much lower affinity than benchmarks (figure 4D).

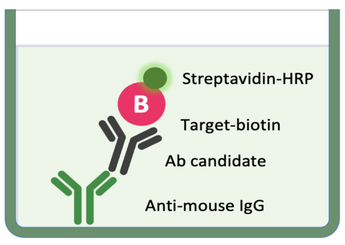

Figure 4A

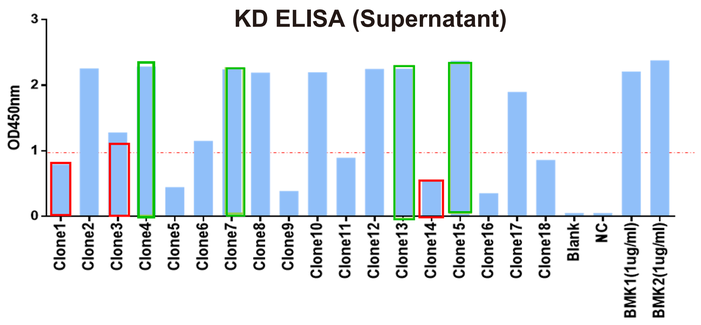

Figure 4B

FACS Binding-CHO-K1/CD117 cell pool

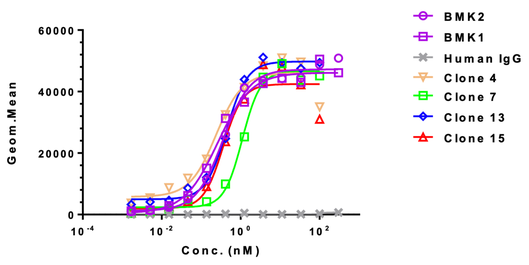

Figure 4C

FACS Binding-CHO-K1/CD117 cell pool

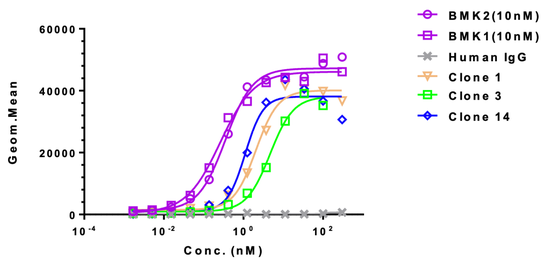

Figure 4D

2. Reporter Gene Assay (RGA) using *Powerdoma™* supernatant

TIGIT reporter gene assay was conducted with ***powerdoma™*** supernatant (figure 5A), identifying four potential functional blockers of TIGIT/CD155 signaling (clone 3, 7, 15, 19, in green box), and one non-functional binder (clone 10, in red box). These candidates were recombinantly expressed and the purified Abs subjected to full curve blocking assays. As shown in figure 5B, these previously identified functional blockers are confirmed to potently block TIGIT/CD155 signaling, while the non-functional binder (clone 10) binds to TIGIT (figure 5C) without signaling blockade (figure 5D).

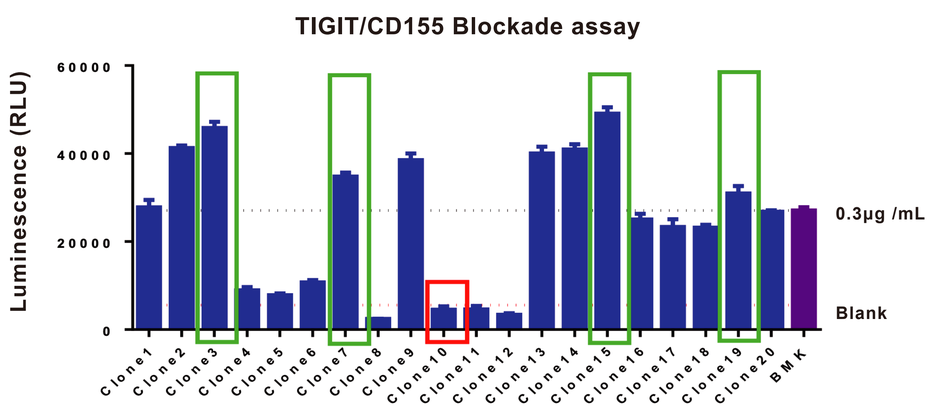

Figure 5A

TIGIT/CD155 blockade assay

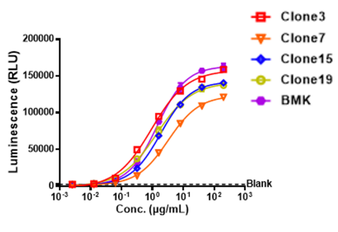

Figure 5B

FACS Binding-CHO-K1/Human TIGIT stable cells(+)

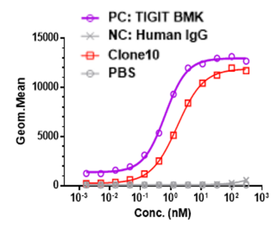

Figure 5C

TIGIT Blockade bioassay on Opti-TIGIT effector cell+ GS-C2/CD155

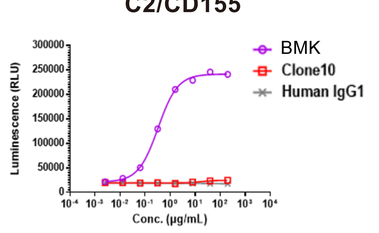

Figure 5D

GPCR assay using *Powerdoma™* supernatant

cAMP assay is a well-recognized approach to evaluate the agonist /antagonist activity of antibody against GPCR targets. By such an assay using ***powerdoma™*** supernatant (figure 6A), a number of potential antagonist Abs against target A were identified. Consistently, after recombinant expression and purification, these Abs were confirmed to block target A mediated cAMP production in a dose-dependent manner (figure 6B).

Neutralization Assay on CHOK1/Gα15/GPCR target A

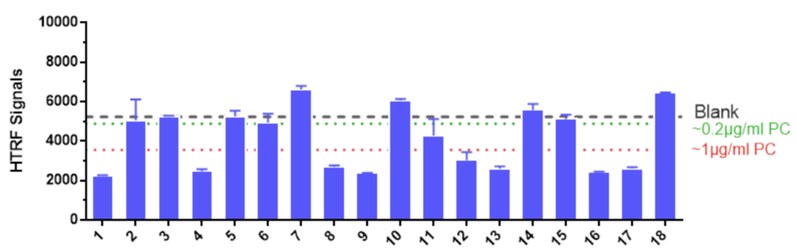

Figure 6A

Neutralization Assay on CHOK1/Gα15/GPCR target A

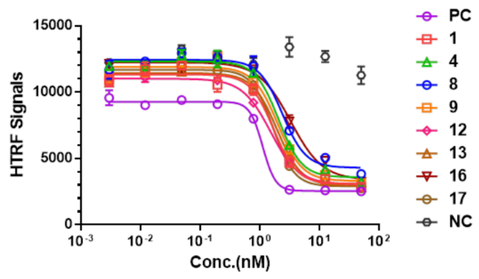

Figure 6B

CONCLUSION

Taken together, the ***Powerdoma™*** workflow is an upgraded hybridoma platform for Ab lead discovery, with substantially shortened timeline and improved cost-effectiveness. More importantly, it makes it feasible to conduct functional screening at very early stage of Ab lead discovery, which may greatly improve the chance of success in discovering functional leads with desired target product profile.

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