Rapid Lead Generation with the Pioneer Antibody Discovery Platform

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Introduction

The Pioneer™ Antibody Discovery Platform is a collection of technologies that forms Bio-Rad's biotherapeutic lead generation service. The Pioneer Antibody Library is central to the Discovery Platform. Its design is informed by over 18 years of experience in antibody phage display. It was engineered to have optimal properties for the selection of therapeutic antibody candidates, including the reduction of CDR-located posttranslational modification sites for improved developability. With 89% of the clones (VH and VL combined) encoding functional antibodies, the Pioneer Antibody Library encodes 2.2×10^{11} unique antibodies and is one of the largest functional Fab antibody libraries ever made. The Pioneer Platform takes advantage of SpyDisplay, our proprietary selection system based on SpyTag technology. Selected Fab candidates can be directly used with Bio-Rad's modular antibody assembly platform, TrailBlazer™, allowing fast and easy screening in a variety of formats, including various IgG-like formats. We present data on antibody selection against IL-6R, C5aR, and CXCR4, demonstrating that the Pioneer Platform yields diverse, high-affinity, and functional leads — even for difficult targets like GPCRs. These candidates show comparable or superior performance to clinically developed or marketed antibodies across key parameters.

Pioneer Library Design: Maximized Use of Library Space, Limited Liabilities

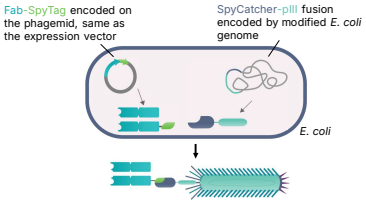
The Pioneer library is a synthetic human Fab library.
Highly curated for:

- Ideally suited sequences for phage display and *E. coli* expression
- Germline genes with known beneficial properties that are frequently found in therapeutic antibodies
- Consists of 4 sublibraries VH1k, VH1l, VH3k, VH3l
- Germline-specific CDR design, all 6 CDRs diversified
- Strongly reduced potential PTM sites and other liability motifs
- SpyTag built in for antibody display and antibody format switching

Functional library size is 2.2×10^{11} unique antibodies, one of the largest functional antibody libraries.

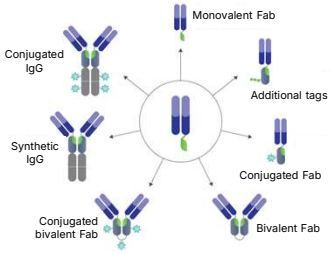
SpyTag Built in for Accelerated Selection and Characterization

SpyDisplay



SpyTag protein ligation occurs inside the *E. coli* and results in a covalent display of the Fab.

TrailBlazer Antibody Platform



Advantages of SpyDisplay:

- Rapid 1 day/panning round protocol and fewer errors as no subcloning step required
- Monovalent display system capable of selecting antibodies with sub nM affinities
- Resulting antibodies carrying a SpyTag, compatible with TrailBlazer Antibody Platform

Faster selection process for high-affinity antibody discovery.

Advantages of TrailBlazer:

- Simplest protocol
- 1 hr reaction
- No purification
- Fully scalable
- Improved performance
- Accelerated workflow
- Economical

Lead Candidate Discovery: Excellent Affinities and Diversity

IL-6R

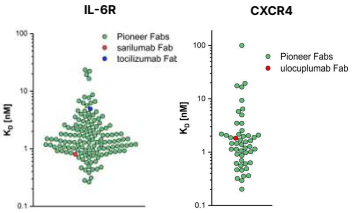
- Target: extracellular domain of IL-6RA, immobilized on beads
- 144 unique antibodies
- 27% have sub nM affinities, 97% <10 nM

C5aR Collaboration with SVAR

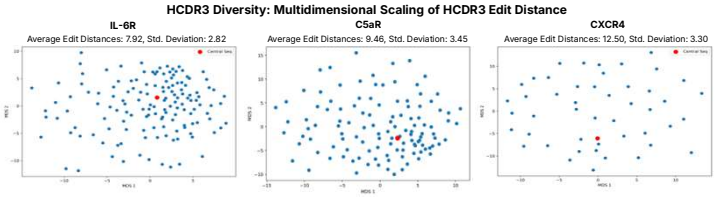
- Target: cell panning on C5aR overexpressing cells from SVAR
- 119 unique antibodies

CXCR4 Collaboration with SALIPRO BIOTECH

- Target: Purified stabilized CXCR4 nanodiscs from Salipro
- 48 unique antibodies
- 33% have sub nM affinities, 92% <10 nM



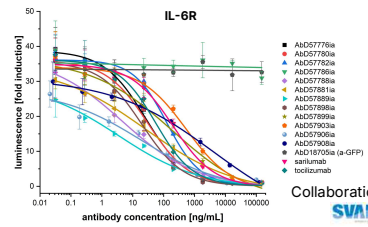
HCDR3 Diversity: Multidimensional Scaling of HCDR3 Edit Distance



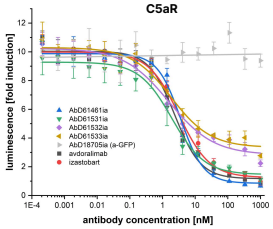
No CDR clusters in multidimensional scaling plots.

Functionality of IgGs in Reporter Cell Assays — IL-6R and C5aR

Inhibition of IL-6R and C5aR signaling using SVAR's iLite cell assays, in which engineered cells express luciferase upon stimulation with IL-6 or C5a. Benchmarks were used as positive controls, and AbD18705ia Anti-GFP IgG1 as a negative control. Results represent induction fold over sample treated with no antibody. For IC_{50} values see tables on the right.

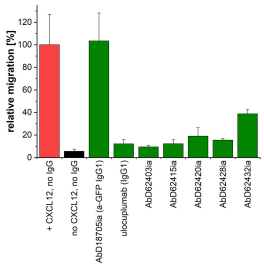


Collaboration with SVAR



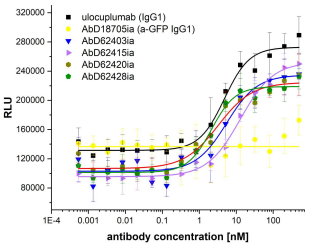
Functionality of IgGs in Cellular Assays — CXCR4

Anti-CXCR4 antibodies demonstrated comparable inhibition of cell migration to ulocuplumab.



Inhibition of CXCR4-dependent migration of Jurkat cells in a transwell assay. Cells were preincubated in 50 nM of the indicated antibodies and their migration to the compartment containing high concentration of CXCL12 was quantified and expressed as percentage of the positive control, which was not treated with any antibody. Ulocuplumab (in IgG1 format) and AbD18705ia Anti-GFP IgG1 were used as positive and negative controls, respectively.

Antagonistic CXCR4 antibodies deactivated adenylate cyclase in a concentration-dependent manner.



Assay for CXCR4-dependent deactivation of adenylate cyclase. Cells were preincubated with serially diluted antibodies, adenylate cyclase was activated with forskolin and CXCR4 by CXCL12 addition, followed by cell lysis and quantification of cAMP. High signals represent high cAMP levels and therefore high activity of adenylate cyclase, i.e., an efficient block of CXCR4 signaling by the antibodies. Ulocuplumab and AbD18705ia Anti-GFP IgG1 were used as positive and negative controls, respectively.

IgG Lead Candidate Selection

IL-6R

antibody	monovalent affinity [nM]	IC_{50} in cellular assay [ng/mL]	Tm1 [°C]	Tm2 [°C]	SEC monomer content	SI-BLI signal [nm]	HIC main peak retention time [min]	stability (4 weeks, 37°C)	poly-reactivity test	germline identity	
										VL	VH
sarilumab	0.25	199	64.8	80.3	98%	0.30	4.7	n.d.	passed	97%	95%
tocilizumab	1.26	55	68.8	80.5	99%	0.32	5.2	n.d.	passed	90%	83%
AbD57776	0.22	26	64.9	80.5	99%	0.37	10.9	stable	passed	91%	91%
AbD57780	0.24	31	65.2	76.9	97%	0.46	7.0	stable	passed	94%	95%
AbD57782	0.06	126	65.2	78.1	100%	0.24	4.8	stable	passed	95%	92%
AbD57881	0.22	26	64.2	71.1	98%	0.43	10.7	stable	passed	90%	96%
AbD57898	0.24	19	64.7	77.8	97%	0.32	5.4	stable	passed	96%	94%
AbD57899	0.36	195	64.8	79.1	100%	0.32	4.8	stable	passed	96%	93%
AbD57903	0.58	532	65.0	80.4	100%	0.32	6.2	stable	passed	96%	91%
AbD57906	0.25	19	60.9	81.1	100%	0.45	10.3	stable	passed	89%	97%

C5aR

antibody	flow staining EC_{50} [nM]	IC_{50} in cellular assay [nM]	epitope bin	Tm1 [°C]	Tm2 [°C]	SEC monomer content	SI-BLI signal [nm]	HIC main peak retention time [min]	poly-reactivity test	germline identity	
										VL	VH
avdoralimab (IgG1)	2.0	2.4	A	66.1	68.9	100%	0.45	6.9	passed	99%	94%
izastobart (IgG1)	2.0	3.2	B	63.2	77.9	99%	0.39	5.6	passed	90%	91%
AbD61461	2.6	3.6	A	64.6	77.0	96%	0.81	9.7	passed	91%	94%
AbD61531	0.5	1.9	B	62.8	76.0	96%	0.54	6.0	passed	87%	92%
AbD61532	0.4	1.6	B (C?)	66.6	66.6	99%	0.47	6.4	passed	89%	94%
AbD61533	1.0	1.9	B	65.3	74.0	100%	0.46	5.6	passed	91%	92%

CXCR4

antibody	monovalent affinity [nM]	migration at 50 nM IgG [%]	Tm1 [°C]	Tm2 [°C]	SEC monomer content	SI-BLI signal [nm]	HIC main peak retention time [min]	poly-reactivity test	germline identity	
									VL	VH
ulocuplumab (IgG1)	2.39	12	68.4	77.0	98%	0.43	6.7	passed	99%	97%
AbD62403	0.39	9	64.6	78.2	99%	0.62	6.0	passed	95%	98%
AbD62415	0.20	12	64.8	72.8	99%	0.68	8.2	passed	96%	98%
AbD62420	1.33	19	64.7	78.4	97%	0.59	8.3	passed	92%	98%
AbD62428	0.73	15	64.4	69.5	93%	0.66	5.9	passed	89%	93%

Tm1/2: melting temperatures measured by nanoDSF
SEC: monomer content in size-exclusion HPLC
SI-BLI: self-interaction [nm] assessed by BLI, normalized to sensor loading
HIC: retention time [min] in hydrophobic interaction chromatography
Polyreactivity assessed by binding to dsDNA and insulin
Stability assessed by analyzing monomer content by SEC and band pattern by capillary electrophoresis
Germline identity: amino acid sequence identity compared to the closest germline sequence (from FR1 to FR3)

Summary

The Pioneer Antibody Discovery Platform comprises:

- One of the largest functional phage display antibody libraries ever made
- Minimized sequence motifs known to be problematic for drug developability
- SpyTag built in:
  - Proprietary SpyDisplay selection technology for rapid generation of high-affinity antibodies
  - TrailBlazer technology accelerates screening and characterization in various formats
  - SpyLock technology for high-throughput bispecific prototype production

We successfully completed three antibody discovery programs targeting IL-6R, C5aR, and CXCR4, demonstrating the robustness of the Pioneer Platform:

- Large number of unique antibodies after selection
- ~25% of antibodies exhibited subnanomolar affinities
- High sequence diversity with broad epitope coverage
- Functionally active antibodies with potencies comparable to clinical-stage candidates
- Favorable developability profiles

These results highlight the ability of the Pioneer Library to directly yield therapeutic leads with properties on par with antibodies currently in clinical development or on the market.

The work in this poster was published in *mAbs*

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