SuperHuman Library 2.0

Hits against any epitope
* 76 billion antibodies
* >5000 hits against every target panned
* >300 picomolar hits against each antigen
* Unprecedented, fully-natural CDR diversity
* Computationally optimized CDR fitness

Superior encoded developability
* Drug-worthy scaffolds
* Naturally selected CDR diversity
* 100% germline frameworks
* enhanced thermostability
* Depleted liabilities

Revolutionary Optimization
* single-pass multi-parameter optimization
* easy affinity maturation
* easy cross-species optimization
* agile vector system

A decade of computational immunology Big Data distilled into a revolutionary antibody library
High-throughput repertoire sequencing has revolutionized antibody library design.

Hits against PCSK9

2009 Natural
Glanville & Zhai PNAS 2009

2011 Naïve

2011 Synthetic
Zhai & Glanville JMB 2011

2013 Synthetic
Monoclonal lead discovery & development time, in months

- Hybridoma: 5-20 Hits
- Traditional Phage: 5-50 Hits
- Transgenic: 5-20 Hits
- SuperHuman+Carterra: 5000-9000 Hits
- SuperHuman Zero-Day: 96 Hits

Steps:
- Immunization
- Hybridoma
- Panning
- Screening
- Sequencing
- Humanization
- Optimization
Primary screen of 2 96-well plates of clones randomly selected after round 4 SuperHuman panning of PD1.

Samples immediately assayed on Carterra high-throughput kinetics instrument, bypassing ELISA screening.

Majority of hits were positive and of 184 sequenced, 98 were unique.
Primary screen of SuperHuman Round 4 hits against PD1
Primary screen of SuperHuman Round 4 hits against PD1
Confirmed reactivity against human and cyno cell surface PD1

controls

selected clones

human

cynomolgus monkey

counts

counts

counts

fluorescence intensity
Thousands of hits = antibodies with remarkable properties

Example 1: in an unprecedented feat, we have generated 10 anti-PD1 hits with cross-reactivity to human, primate and mouse on cell surface

12.6nM

Human

Mouse

Cyno

101.8nM

Human

Mouse

Cyno
Rapidly screen for ligand blockade

Screen 384 anti-V5-captured anti-GHR scFv’s in parallel for ligand blockade by injecting:

(1) Premixed GHR/GH
(2) GHR followed by GH ligand:

Bin into:
- Blockers
- Non-blockers
- Displaced by ligand (nuanced blockade)
bGal ELISA & Sanger screening: 2 plates = 61 positives = 49 unique clones
Sanger screening: hits span variation in all frameworks and all CDRs

A robust population of unique hits helps ensure that every epitope of interest is targeted. Extreme diversity of round 3 outputs ensure that hits against any epitope can be recovered by screening a few 96-well plates of clones.
>5000 hits against every antigen (NGS sequencing of Round 3 panning selections)
SuperHuman pre-optimizes hits

While the CDR-H3 and the V-gene frameworks define much of a clone’s interaction with target, variations in the other CDRs can impact affinity and breadth of reactivity. Our library was designed with about 5 billion CDR-H3s but 76 billion total antibodies. As a consequence of this combinatorial design, each hit will appear with multiple variants, providing key engineering guidance and optimization of every hit right out of the library.
SuperHuman 2.0: Superior Scaffold Selection

- Good libraries need multiple scaffolds to target all epitopes
- However, only some scaffolds are developable
- Distributed Bio applies a 6-fold analysis to identify a final collection of optimal VH and VL scaffolds

<table>
<thead>
<tr>
<th>IGHV4-34</th>
<th>half-life</th>
<th>inherently autoreactive to blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGHV2-5</td>
<td>stability</td>
<td>inferior stability profile</td>
</tr>
<tr>
<td>IGHV4-b</td>
<td>immunogenicity</td>
<td>V-gene not found in 50% of individuals</td>
</tr>
<tr>
<td>IGLV6-57</td>
<td>aggregation</td>
<td>Aggregation-prone V-gene</td>
</tr>
</tbody>
</table>

**TABLE 1:** Example of problem frameworks to be avoided
Phase I+: Only a subset of the natural repertoire has been through human trials

An analysis of scaffold usage in >400 mAbs from Phase I+

Most human scaffolds have never been used for a mAb

Almost all Phase mAbs are kappa-derived
Display fitness: only a subset of the natural repertoire displays well on phage

Traditional natural libraries: only 37% functional fitness

Glanville et al, 2009 PNAS
Immunogenicity: only some V-genes and alleles are shared by all human populations
SuperHuman 2.0 – Combining design & selection for optimal fitness

- In most libraries, over 90% of light chains aren’t in frame, don’t express or aren’t stable
- These poor light chains reduce the size of the library by “poisoning” even functional VH partners
- In SuperHuman v2 we apply functional selection for expression and thermostability during construction to produce a library with over **95% functional diversity** across **40 million light chains**
- We then load our optimal VH diversity and transform a 7.6e10 library with superior functional fitness

**Build VK library** → **Display VK library** → **Heat stress (>65C)** → **protein A/L select** → **Load VH diversity**

Lose garbage and poor expression

Lose unstable and aggregation prone material

Lose protein A/L non-binders
Traditional natural library VHs are limited to 1e7 due to redundancy of blood-derived antibody diversity.

SuperHuman design enables a fully human library to achieve >1e11 diversity in the VH.

Library redundancy: accumulative frequency of most abundant clones

- 2% redundancy @ 100k depth
- 70% redundancy @ 10k depth

Most abundant VH clones, ranked by frequency

- Glanville, PNAS 2009
- Zhai & Glanville, JMB 2011
- Mohan, JMB 2011
- Glanville, COSB 2015
SuperHuman 2.0 Replicate clone overlap

VH replicate A
3,692,953 sequences
98.5% unique clones

VH replicate B
3,901,603 sequences
98.7% unique clones

overlap
4,932 sequences

VH 99.93% unique clones

VK replicate A
1,061,309 seqs
72.1% unique

VK replicate B
1,101,829 seqs
72.3% unique

overlap
79,885 sequences

VK 94.9% unique VK clones
SuperHuman Zero-Day Discovery: Pre-Panned Targets

- PD1
- LAG3
- OX40
- CTLA4
- SIRPa
- CD47
- VISTA
- 41BB
- TIM3
- GITR
- ICOS
- TIGIT
- GHR
- HGH
- amyloid beta
- alpha synuclein
- Tau
- Beta secretase

...
Tumbler biases SHM to preferentially explore the near-germline space

- Single mutants (~150)
- Double mutants (~20,000)
- Triple mutants (~3,000,000)
- Quadruple mutants (~500,000,000)

\[ f_i = \text{Prob}(i) = \text{Prob}(\lambda_i) \times \prod_{p=1}^{\lambda_i} \text{Prob}(\alpha_{i,p} | \text{PFM}_\lambda) \]

Tumbler libraries are preloaded affinity maturation of a billion lineages into SuperHuman framework CDRs
Distributed Bio SuperHuman 2.0 (Q1 2017)

**Scaffolds:** 4VH (HV1-46, HV1-69, HV3-15, HV3-23)  
4VL (KV1-39, KV2-28, KV3-15, KV4-1)  
100% germline, stable, proven, non-immunogenic, aggregation resistant, & phage-compatible

**Diversity:** 7.6e10 transformants. CDR-H3 and CDR-L3 from CD27-/IgM+ naïve B-cells from >140 donors. Other CDRs from CD27+ memory B-cells from >140 donors, framework specific

**Tumbler:** Generate 100,000,000+ versions of your antibody in a day  
Perform computational optimization with AbGenesis

**Subscription:** new scaffolds/updates in 2018 3.0

**AbGenesis:** Integrated computational tools to facilitate screens and optimizations of hits

“We are loving your library” – a happy partner