Combining SuperHuman-2.0 and Carterra LSA
to realize the dream of a one-week antibody discovery cycle
Bioengineering: 9-12 months
Human body: 7 days

Bioengineering: 9-12 months
Hybridoma

Traditional Phage

Transgenic

Monoclonal lead discovery & development time, in months

Hits

5-20

5-50

5-20
Hybridoma lead discovery & development time, in months

- **Hybridoma:**
  - Immunization
  - Hybridoma
  - Panning
  - Screening
  - Sequencing
  - Humanization
  - Optimization

- **Traditional Phage:**
  - Immunization
  - Hybridoma
  - Panning
  - Screening
  - Sequencing
  - Humanization
  - Optimization

- **Transgenic:**
  - Immunization
  - Hybridoma
  - Panning
  - Screening
  - Sequencing
  - Humanization
  - Optimization

- **SuperHuman+Carterra:**
  - Immunization
  - Hybridoma
  - Panning
  - Screening
  - Sequencing
  - Humanization
  - Optimization

- **SuperHuman Zero-Day:**
  - Immunization
  - Hybridoma
  - Panning
  - Screening
  - Sequencing
  - Humanization
  - Optimization

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**Hits**

- **Hybridoma:** 5-20
- **Traditional Phage:** 5-50
- **Transgenic:** 5-20
- **SuperHuman+Carterra:** 5000-9000
- **SuperHuman Zero-Day:** 96
High-throughput repertoire sequencing has revolutionized antibody library design

Nanomolar affinity (KD)

Hits against PCSK9

2009 Natural
Glanville & Zhai PNAS 2009

2011 Naïve

2011 Synthetic
Zhai & Glanville JMB 2011

2013 Synthetic
Distributed Bio presents

SuperHuman 2.0

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

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

A decade of computational immunology Big Data distilled into a revolutionary antibody library
SuperHuman 2.0:
Scaffold Selection
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>IGTV4-34</th>
<th>half-life</th>
<th>inherently autoreactive to blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGTV2-5</td>
<td>stability</td>
<td>inferior stability profile</td>
</tr>
<tr>
<td>IGTV4-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

IGHV1-17  IGHV1-18  IGHV1-2  IGHV1-24  IGHV1-3  IGHV1-46  IGHV1-48  IGHV1-69

IGHV2-26  IGHV2-5  IGHV2-70

IGHV3-11  IGHV3-13  IGHV3-15  IGHV3-20  IGHV3-21  IGHV3-22  IGHV3-23  IGHV3-43  IGHV3-48  IGHV3-49  IGHV3-64  IGHV3-7  IGHV3-72  IGHV3-73  IGHV3-74

**Allele**

- *01
- *02
- *03
- *04
- *05
- *06
- *07
- *09
- *10
- *12
- *13
- *d

Counts

Subpop


*IGHV1-69 has multiple alleles, but all variation is found in CDRs and is therefore permitted*
Aggregation resistance: some V-genes are aggregation-prone
Thermostability: some V-genes are more stable than others

Family Fab thermostability variation (Tm kJ/mol)

VH1a 13.7
VH1b 26
VH3 52.7
VH5 19.1
Vk1 29
Vk2 24.8
Vk3 34.5
Vl1 23.7
Vl2 16
Vl3 15.1

Biophysical Properties of Human Antibody Variable Domains, Ewert, Huber, Honnegar, Pluckthun
Structural diversity: most human V-genes are structurally redundant

SuperHuman is built from 16 structurally non-redundant scaffolds that explore 16 unique canonical paratope topologies
<table>
<thead>
<tr>
<th>IGHV1-46</th>
<th>canon:1-3  IGHV1-2, IGHV1-3, IGHV1-8, IGHV1-45, IGHV1-58</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>abituzumab, amatuximab, bavituximab, benralizumab, bococizumab, burosumab, cabirilumab, codrituzumab, coltuximab, daclizumab, dinutuximab, eculizumab, emicizumabanti-factorx, ficolatuzumab, futuximab, ibalizumab, ibritumomab, indatuximab, inotuzumab, isatuximab, laprituximab, lendalizumab, matuzumab, mirvetuximab, muromonab-cd3, nemolizumab, ozanezumab, pogalizumab, ponezumab, ralpancizumab, ruplizumab, setoxaximab, suvizumab, tenatumomab, tildrakizumab, tositumomab, ublituximab, veltuzumab, visilizumab, zatuximab</td>
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</table>

<table>
<thead>
<tr>
<th>IGHV1-69</th>
<th>canon:1-2  IGHV1-18, IGHV1-F</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>belimumab, blinatumomab, carlumab, cixutumumab, diridavumab, enokizumab, epratuzumab, firivumab, fontolizumab, fresolimumab, galcanezumab, gemtuzumab, imgatuzumab, ixekeilizzumab, ligeluzumab, lirilumab, nimotuzumab, obinutuzumab, rafivirumab, risankizumab, rontalizumab, simtuzumab, solitomab, tadocizumab, tesidolomab</td>
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</table>

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<thead>
<tr>
<th>IGHV3-15</th>
<th>canono:1-U  IGHV3-49</th>
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<td>inebilizumab, tregalizumab</td>
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<tr>
<th>IGHV3-23</th>
<th>canon:1-3  IGHV3-30, IGKV3-7, IGHV3-48, IGHV3-74, IGHV3-21, IGHV3-9, IGHV3-11, IGHV3-64, IGHV3-43, IGHV3-20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alirocumab, anrkinzumab, bapineuzumab, bevacizumab, certolizumab, daratumumab, denosumab, domagrozumab, dupilumab, elgemtumab, emicizumabanti-factorix, gantenerumab, imalumab, lanadelumab, landogrozumab, ocrelizumab, opicinumab, orticumab, otelixizumab, oxelumab, polatuzumabvedotin, radretumab, ranibizumab, seribantumab, siltuximab, tigatuzumab, tisotumabvedotin, trevogrumab, vandortuzumab, vantictumab, vesencumab</td>
</tr>
<tr>
<td>IGKV1-39</td>
<td>canon:1-1 IGKV1-5, IGKV1-33, IGKV1-9, IGKV1-12, IGKV1-27, IGKV1-8, IGKV1-16, IGKV1-13, IGKV1D-8, IGKV1-6, IGKV1-17, IGKV1D-43, IGKV1D-17, IGKV1D-16 adecatumumab, ado-trastuzumab, aducanumab, ald518, anrakinzumab, benralizumab, bevacizumab, citatuzumab, clazakizumab, dacetuzumab, domagrozumab, duligotumab, ecuлизumab, emactuzumab, emibetuzumab, enavatuzumab, etrolizumab, galcanezumab, gomiliximab, imalumab, lampalizumab, lendalizumab, lifastuzumab, lumiliximab, monalizumab, moxetumomab, namilumab, nemolizumab, ocrelizumab, omalizumab, parsatuzumab, pateclizumab, pidilizumab, pinatuzumab, pogalizumab, polatuzumab, quilizumab, ranibizumab, romosozumab, rontalizumab, tadocizumab, teplizumab, ticilimumab, tildrakizumab, tovetumab, trastuzumab, tremelimumab, veltuzumab, vesencumab, visilizumab</td>
</tr>
<tr>
<td>IGKV2-28</td>
<td>canon:2-1 IGKV2-30, IGKV2-29, IGKV2D-29, IGKV2-24 cantuzumab, dupilumab, lucatumumab, mogamulizumab, obinutuzumab, sevirumab, tenatumomab, zatuximab</td>
</tr>
<tr>
<td>IGKV3-15</td>
<td>canon:1-1 IGKV3-11, IGKV3-NL4 brodalumab, glebatumumab, indusatumb, ligelizumab, rilotumumab</td>
</tr>
<tr>
<td>IGKV4-1</td>
<td>canon:3-1 IGKV2-40 alirocumab, demcizumab, ficlatuzumab, ibalizumab, isatuximab, lebrikizumab, lilotomab, lumretuzumab, margetuximab, mepolizumab, naptumomab, navicixizumab, patritumab, piritoxaximab, refanezumab, setoxaximab, sibrotuzumab, solitomab, suvizumab, tregalizumab, vorsetuzumab</td>
</tr>
</tbody>
</table>
Sublibrary favoritism – like a stock portfolio, place more bets with the best scaffold pairs

14 billion HV1-46/KV1-39

10 billion HV3-23/KV1-39

2-4 billion for each other combination
SuperHuman 2.0:

CDR Diversity Optimization
Individual adults only sample a small part of the antibody repertoire.

- $10^{14}$: Theoretical IgH naïve V(D)J repertoire
- $10^{13}$
- $10^{12}$
- $10^{11}$: Total B-cells in an adult
- $10^{10}$
- $10^{9}$
- $10^{8}$
- $10^{7}$: Non-redundant PBMC Naïve clones
- $10^{6}$
- $10^{5}$: Non-redundant PBMC IgG clones
Non-natural diversity not tolerated in many positions

How the accumulation of small errors poisons a synthetic library
Human CDR-H3 Length 9

Murine CDR-H3 Length 9

Zhai & Glanville et al, “Synthetic antibodies designed on natural sequence landscapes”, Journal of Molecular Biology, 2011
Obtaining optimal CDR diversity from human B-cells with computational immunology

The naturally selected naïve V(D)J rearranged repertoire is >100x more diverse than the memory repertoire, but lacks variation outside of CDR3

Each donor provides...

Memory CD27+
Low VDJ diversity
High SHM diversity

Naïve CD27-/IgM+
High VDJ diversity
No SHM diversity

The class-switched memory repertoire contains great naturally selected SHM diversity in CDR1 and CDR2, but only contains a few hundred thousand clones in the periphery at a time

Glanville, PNAS 2011
140 donors

**Framework specific amplification**

- **Combinatorial Assembly into 100% germline frameworks**

**Individual donor diversity curated by Illumina MiSeq:**
suboptimal samples identified and excluded from library

**Nearly theoretically optimal CDR combinatorics monitored by Illumina MiSeq.**

**VK diversity = 100x nature**
**VH diversity = 2000x nature**

**L1-L2 Library Assembly Efficiency**

**100% Germline Frameworks (blue)**
**SuperHuman CDR diversity (white)**
Biochemical liability reduction

KV3-28 L1 re-engineered synthetically to avoid liabilities

Cysteines removed by selective removal of problem samples

Natural diversity (140 individuals)

SuperHuman 2.0

SuperHuman 2.0 has the benefits of naturally produced CDRs, but has been depleted of liabilities typical of natural libraries.

Light chains: 95% liability free

Heavy chains: reduced natural liabilities
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

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**Diverse VK & single fixed heavy chain (germline HV3-23 O-)**

4e8 ➔ 1e8 ➔ 5e7 ➔ 4e7 ➔ 7.6e10

**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
100% Germline Developable Scaffolds, Optimized Natural CDR Diversity, >95% VK Fitness
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.

- 2% redundancy @ 100k depth!
- 70% redundancy @ 10k depth!
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
  - 99.93% unique VH 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
  - 94.9% unique VK clones
bGal ELISA & Sanger screening: 2 plates = 61 positives = 49 unique clones

Round 3 bGal panning Replicate A - ELISA plate #1

20 hits

Round 3 Gal panning Replicate B - ELISA plate #2

41 hits
Serology Assays

ELISA

HAI

Repertoire sequencing assay

Cell fate tracing

Clonal expansion

Affinity maturation

Class switching

V,D,J Segment bias

V,D,J Allele bias

CDR convergence

Neutralizing motifs

Diversity estimates

<table>
<thead>
<tr>
<th>Patient</th>
<th>12</th>
<th>16</th>
<th>19</th>
<th>20</th>
<th>21a</th>
<th>21b</th>
<th>128</th>
<th>32</th>
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<tbody>
<tr>
<td>Titer</td>
<td>64</td>
<td>9</td>
<td>502</td>
<td>12</td>
<td>32</td>
<td>128</td>
<td>32</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
>5000 hits against every antigen (NGS sequencing of Round 3 panning selections)
SuperHuman Affinity (nM) of anti-PD1 post-round4 clones

Percentage of clones

Affinity (nM), by Carterra
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.
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)
Hybridoma

Traditional Phage

Transgenic

SuperHuman+Carterra

SuperHuman Zero-Day

Monoclonal lead discovery & development time, in months

<table>
<thead>
<tr>
<th>Method</th>
<th>Time (months)</th>
<th>Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybridoma</td>
<td>5-20</td>
<td></td>
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<td>Traditional Phage</td>
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<td>5000-9000</td>
<td></td>
</tr>
<tr>
<td>SuperHuman Zero-Day</td>
<td>96</td>
<td></td>
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</tbody>
</table>
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 libraries are preloaded affinity maturation of a billion lineages into SuperHuman framework CDRs.
Fig1: A heatmap of viral variation from 6500 strains of influenza reveals the conserved broadly neutralizing epitope in the stem of hemagglutinin (red indicates greater conservation)
**Distributed Bio**
Lauren Schwimmer
Sawsan Youssef
David Maurer
Sarah Ives
Devanshi Shanghavi
Christina Pettus
Ray Newland
Valery Chou
Chris Smith
Giles Day

**Carterra**
Yasmina Abdiche & teams

**USF**
Cary Lai
Jennifer Dever

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