COVID-19 Therapeutic Development in Real Time
Roadblocks and Opportunities

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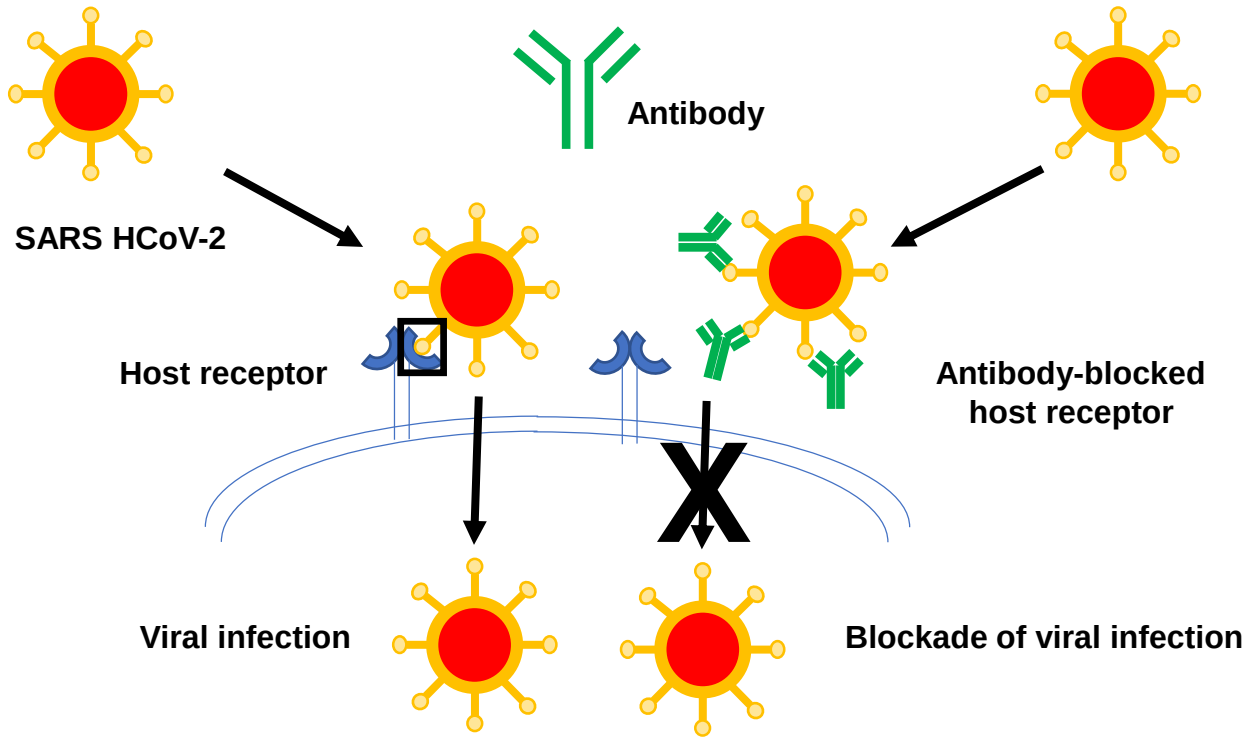
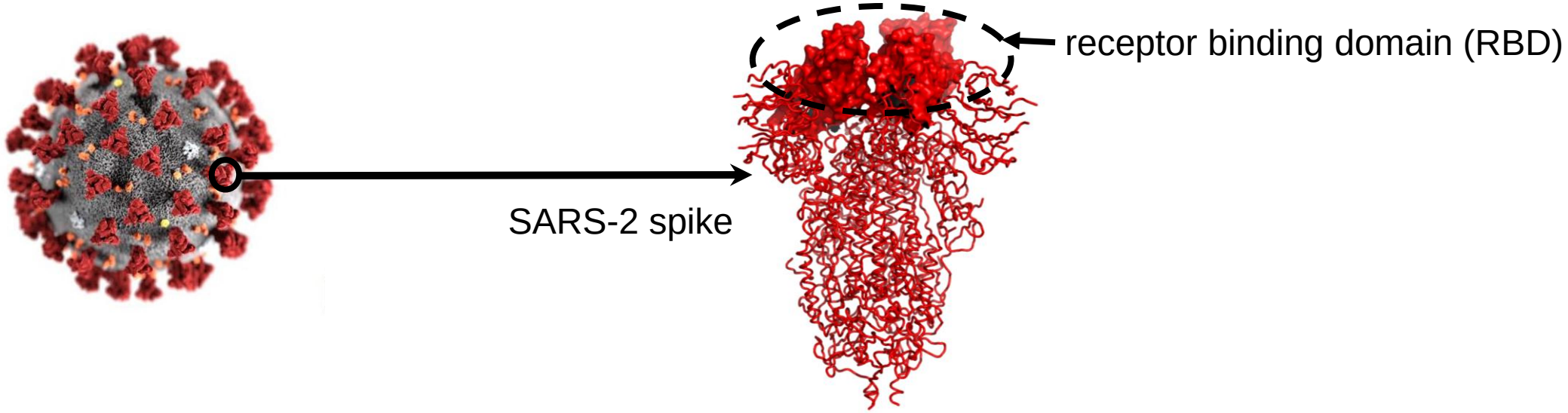
COVID-19 and the Immune System

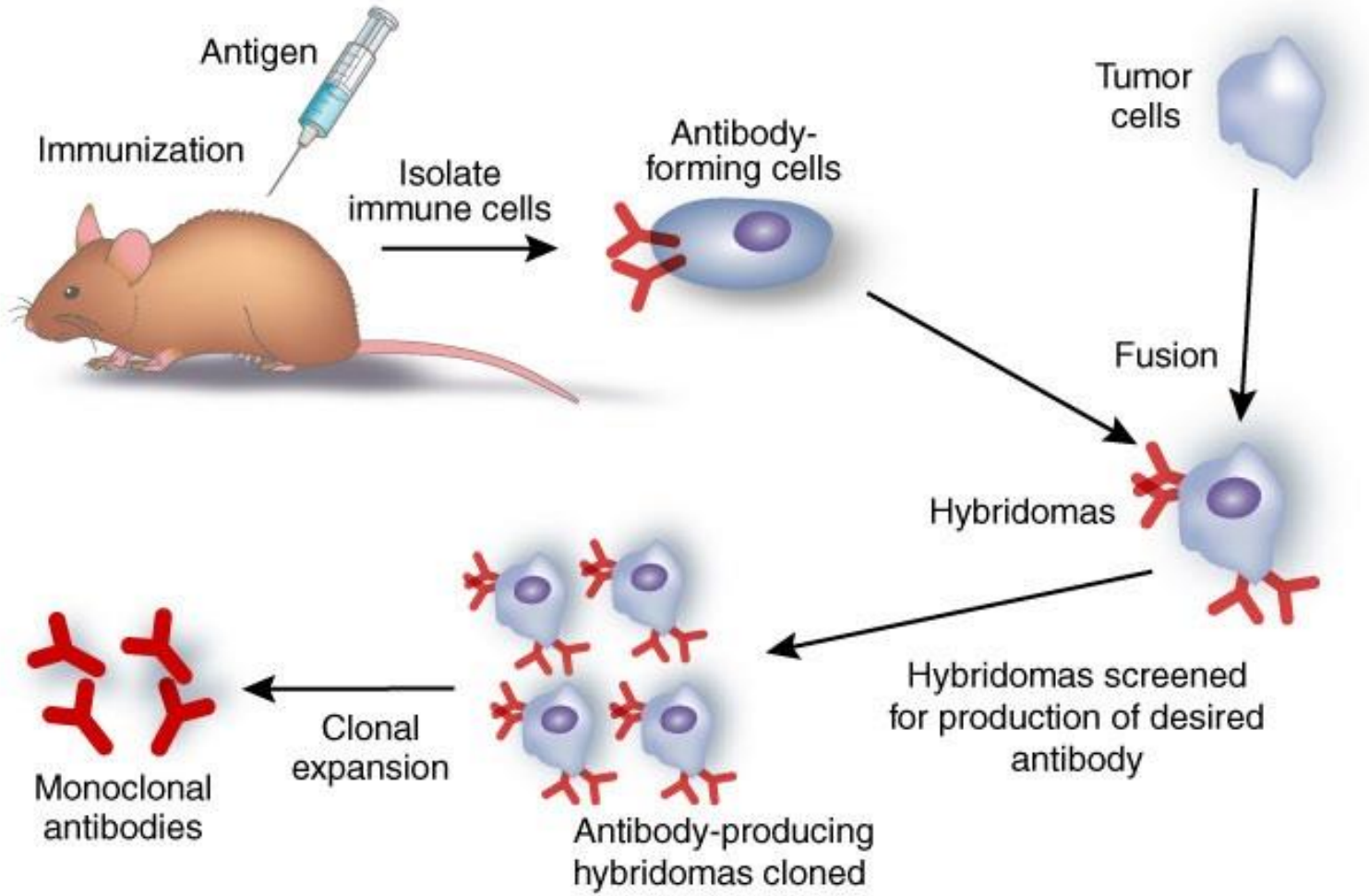


COVID-19 virus SARS-2: Spike infection protein



- **SARS-2 is nearly identical to SARS-1 (80%)**
- **Spike protein mediates host recognition and entry (infection)**
- **RBD recognizes host receptor ACE2**
- **Most natural SARS-1 neutralizing antibodies bind to the RBD and compete for binding with ACE2**
- **Develop synthetic antibodies that bind SARS-2 RBD and compete for binding with ACE2**
- **THESE ARE PRIME CANDIDATES FOR BIOLOGIC THERAPEUTICS FOR COVID19**

Natural Antibodies



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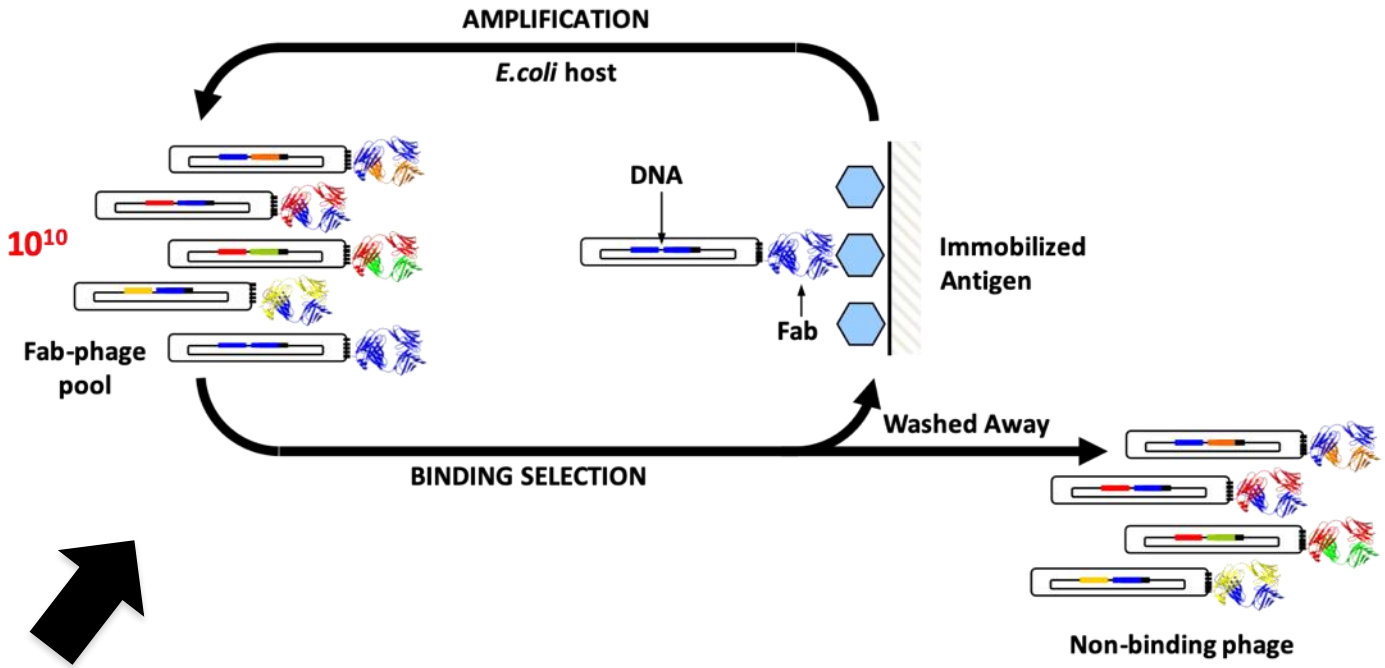
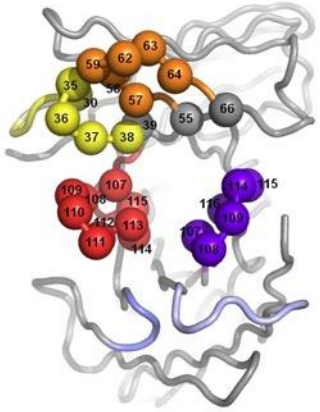
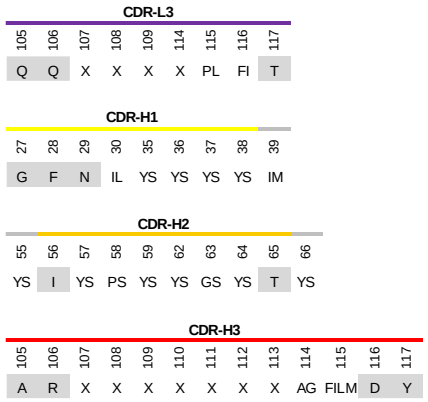
A High-throughput synthetic antibody platform

Highly Validated Technology

- a fully human protein
- Highly stable
- Long half-life (weeks)
- Highly potent
- Highly specific
- Low immunogenicity
- Identical to natural neutralizing antibodies
- **A validated human therapeutic**
  - **(> 50 approved drugs)**







-Single, highly validated human framework (Hereceptin, Avastin, Xolair, etc.)

-High stability and yield, low immunogenicity

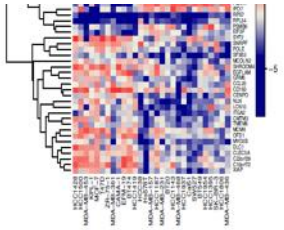
-Minimal targeted synthetic CDR diversity

→Diverse functions with fixed biophysics

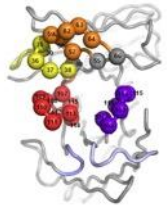
→Modular design

The Therapeutic Antibody War Chest

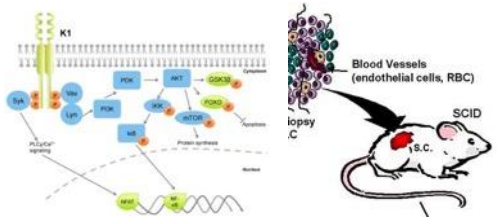
Functional genomics platform



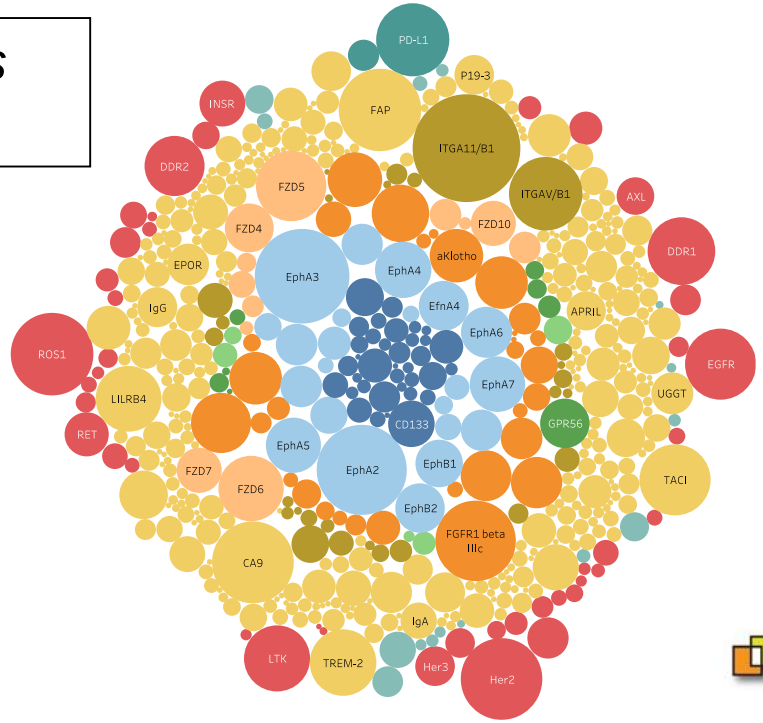
Large-scale, industry-quality antibody generation



Preclinical biology in relevant models



>14,000 Antibodies
>1,300 Antigens



Color Group
- CD
- Ephrin
- FGFR
- FZD
- GPCR
- Ion channel
- ITG
- Other
- PD
- PTP
- RTK

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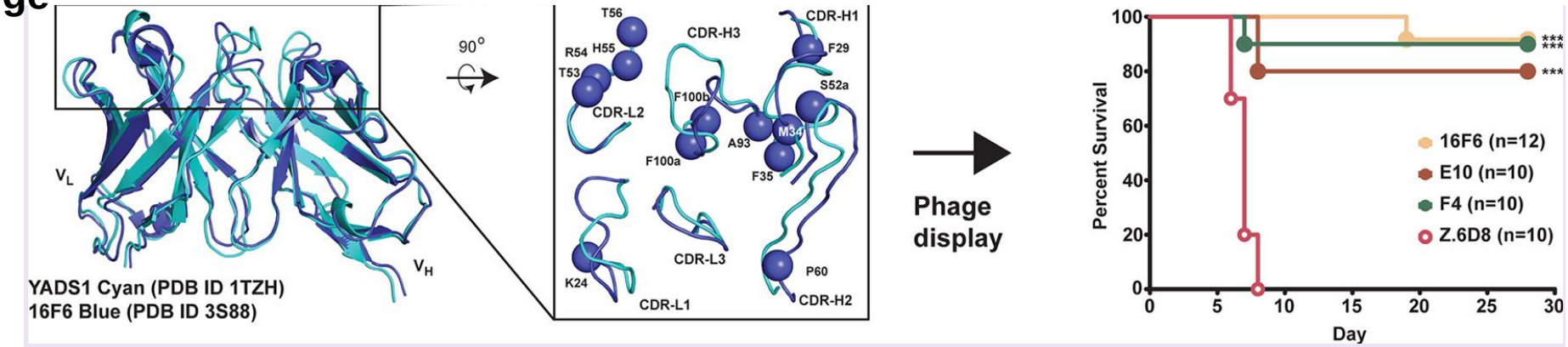
Validated Antibody Platform for Anti-Virals

Synthetic Antibodies with a Human Framework That Protect Mice from Lethal Sudan Ebolavirus Challenge



Sidhu and colleagues:*dx.doi.org/10.1021/cb5006454 | ACS Chem. Biol., 2014*

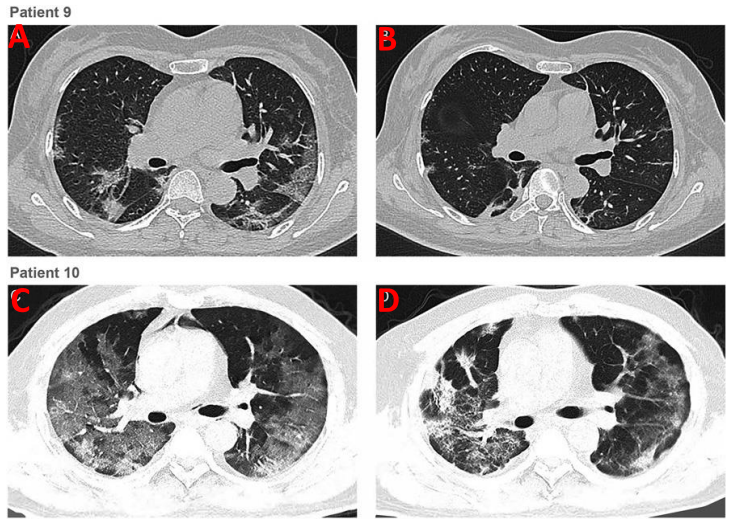
-Similar to SARS-CoV-1/2, Ebola has an acute life cycle
-Developed humanized Abs to key epitope on Ebola virus
-Treatment with single Ab protected virtually all mice from Ebola challenge
-Surviving mice proved resistant to subsequent Ebola challenge
-Ab cleared initial Ebola challenge *and* enabled host immune system to develop natural resistance

Synthetic Neutralizing Antibodies – Proven Stability and Efficacy -Rapid Development and Scalable Cost-effective Production

Effectiveness of convalescent plasma therapy in severe COVID-19 patients

Kai Duan[a,b,1], Bende Liu[c,1], Cesheng Li[d,1], Huajun Zhang[e,1], Ting Yu[f,1], Jieming Qu[g,h,i,1], Min Zhou[g,h,i,1], Li Chen[j,1], Shengli Meng[b], Yong Hu[d], Cheng Peng[e], Mingchao Yuan[k], Jinyan Huang[l], Zejun Wang[b], Jianhong Yu[d], Xiaoxiao Gao[e], Dan Wang[k], Xiaoqi Yu[m], Li Li[b], Jiayou Zhang[b], Xiao Wu[d], Bei Li[e], Yanping Xu[g,h,i], Wei Chen[b], Yan Peng[d], Yeqin Hu[b], Lianzhen Lin[d], Xuefei Liu[g,h,i], Shihe Huang[b], Zhijun Zhou[d], Lianghao Zhang[b], Yue Wang[d], Zhi Zhang[b], Kun Deng[d], Zhiwu Xia[b], Qin Gong[d], Wei Zhang[d], Xiaobei Zheng[d], Ying Liu[d], Huichuan Yang[a], Dongbo Zhou[a], Ding Yu[a], Jifeng Hou[n], Zhengli Shi[e], Saijuan Chen[l], Zhu Chen[l,2], Xinxin Zhang[m,2], and Xiaoming Yang[a,b,2]

www.pnas.org/cgi/doi/10.1073/pnas.2004168117, March 2020



Chest CTs of two patients. **(A)** Chest CT of patient 9 obtained on February 9 (7 dpoi) before CP transfusion (10 dpoi) showed ground-glass opacity with uneven density involving the multilobal segments of both lungs. The heart shadow outline was not clear. The lesion was close to the pleura. **(B)** CT Image of patient 9 taken on February 15 (13 dpoi) showed the absorption of bilateral ground-glass opacity after CP transfusion. (C) Chest CT of patient 10 was obtained on February 8 (19 dpoi) before CP transfusion (20 dpoi). The brightness of both lungs was diffusely decreased, and multiple shadows of high density in both lungs were observed. (D) Chest CT of patient 10 on February 18 (29 dpoi) showed those lesions improved after CP transfusion

FDA Approved a Clinical Trial for Convalescent Plasma Therapy (CPT)

- Patients exhibited strong positive responses within days
-Success of CPT validates neutralizing Abs as effective therapy for COVID19
-Next-generation therapy should be a recombinant neutralizing Ab
-Recombinant Ab will enhance efficacy while obviating limits of CPT
  - -Defined and consistent formulation and activity
  - -Highly stable single agent optimized for neutralization
  - -High purity guarantees high safety compared with undefined plasma

Toronto Recombinant Antibody Centre

A High-throughput synthetic antibody platform **applied to COVID-19**

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- Highly specific
- Low immunogenicity
- Identical to natural neutralizing antibodies
- **A validated human therapeutic**
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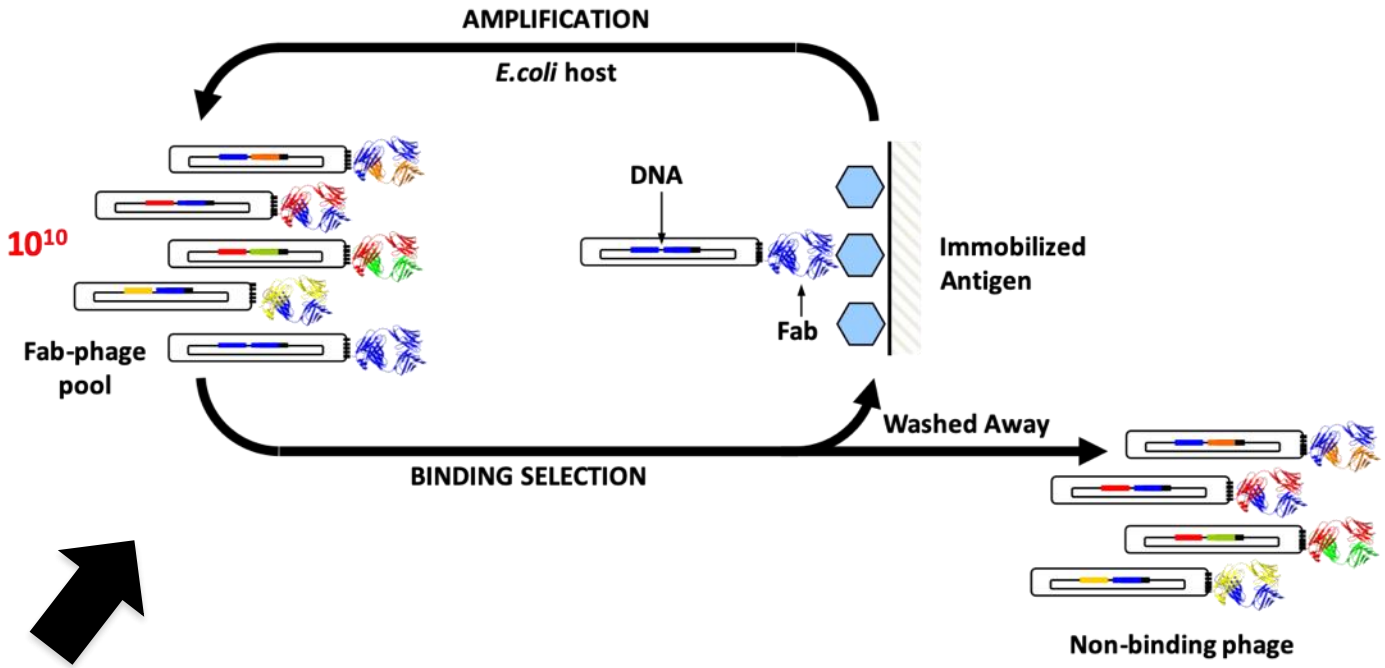

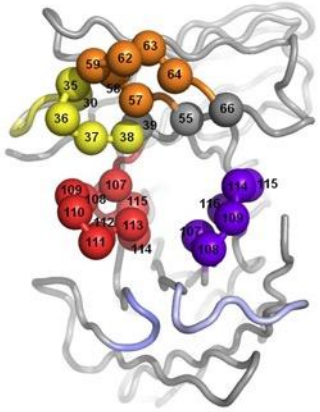

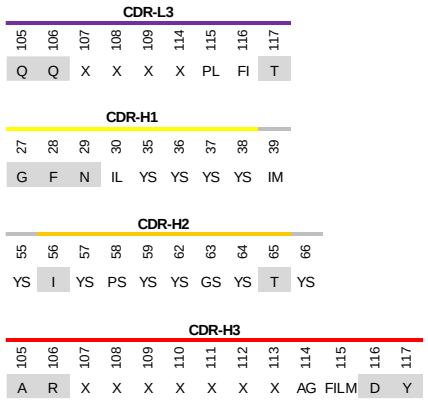

-Single, highly validated human framework (Hereceptin, Avastin, Xolair, etc.)

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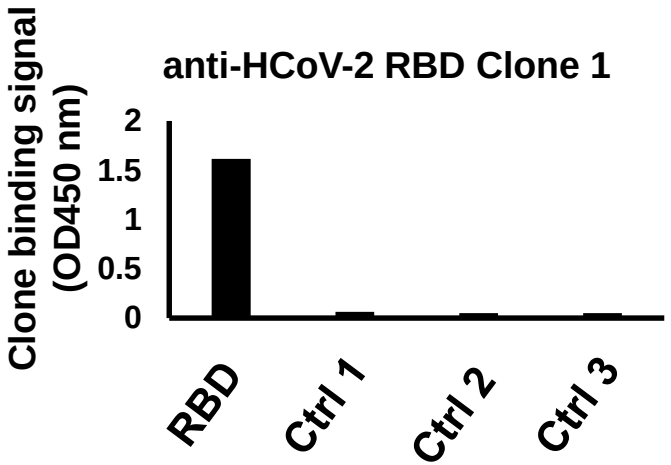
-Minimal targeted synthetic CDR diversity

→Diverse functions with fixed biophysics

→Modular design

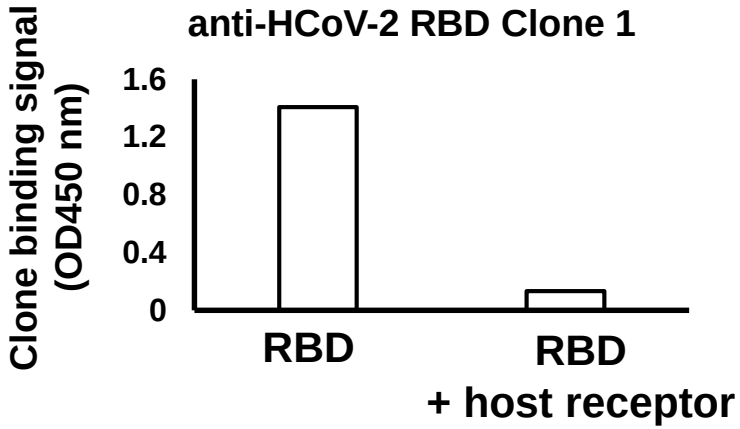
Antibodies bind and block

Binding ELISA



- Binding of most clones to SARS-2 RBD is strong and specific
- 384 clones tested
- 358 positive clones

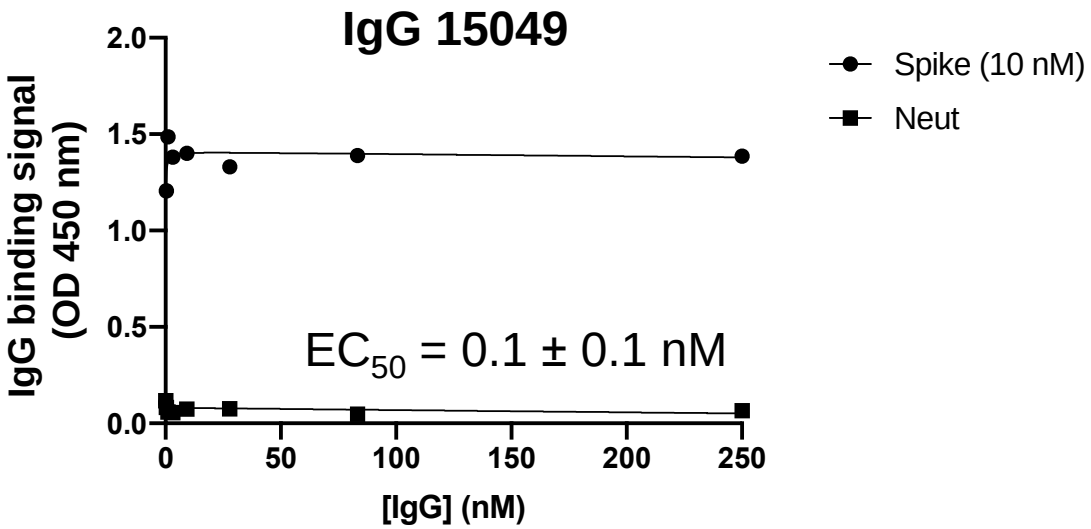
Blocking ELISA



- Binding of many clones is blocked by host receptor
- 358 clones tested
- 66 positive clones exhibit blocking activity
- Clones that exhibit this behaviour as IgG will be the candidate neutralizing antibodies

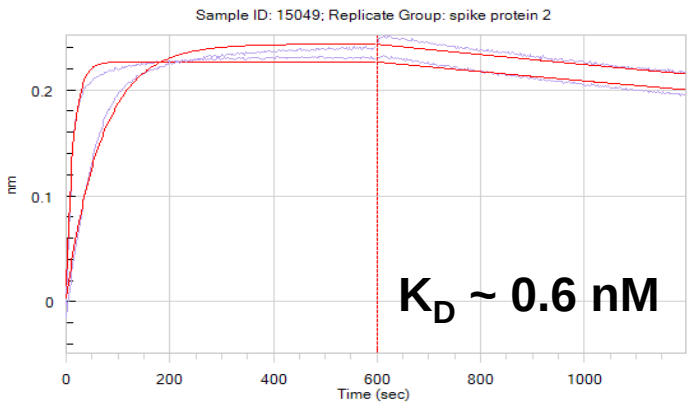
Antibodies are drug-like

EC_{50} affinity estimation by ELISA

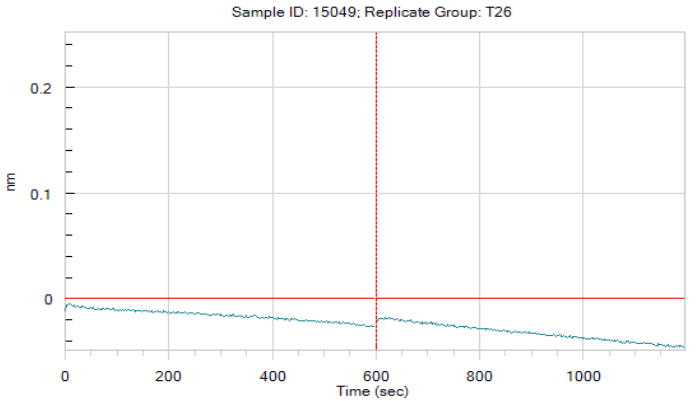


Binding kinetics by BLI

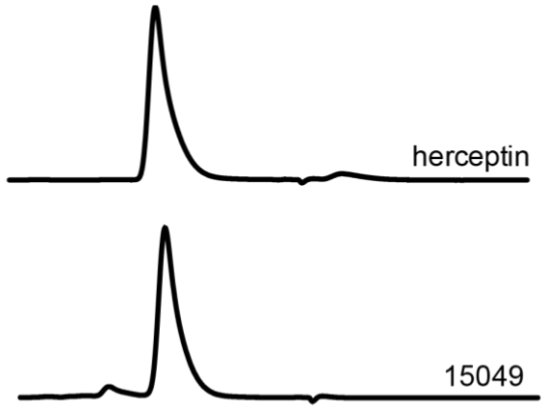
IgG 15049 vs SARS2 spike



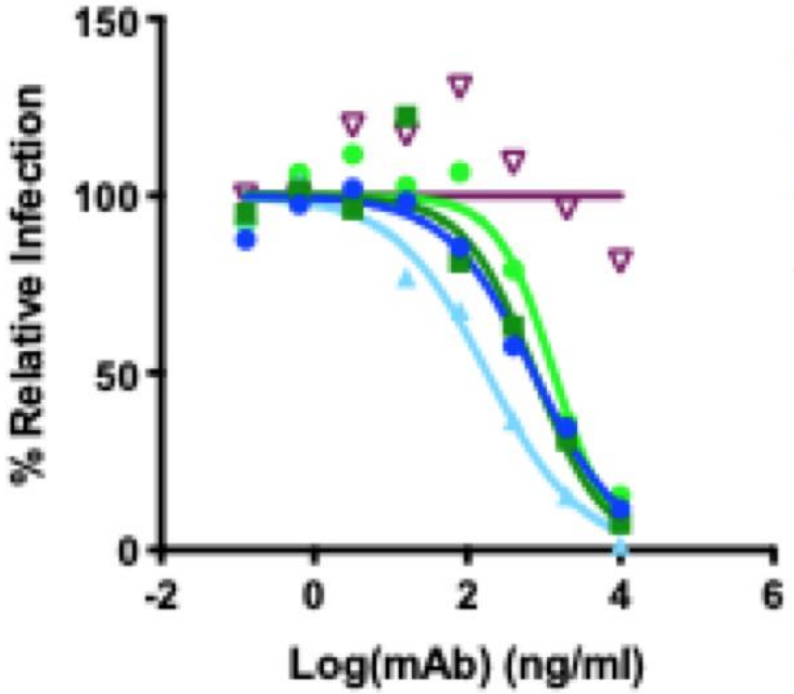
IgG 15049 vs T26 ctrl



SEC profile versus Herceptin standard



Antibodies are Potent Anti-viral Drugs



IgG 15033 Derivatives with Optimized Light Chain

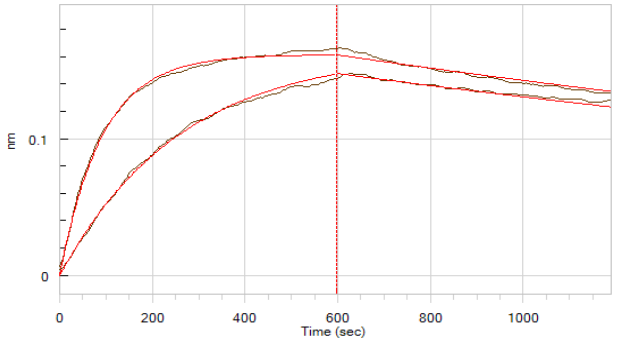
Optimized variants with two substitutions in CDR-L3

Maintain high yield, drug-like SEC, stability

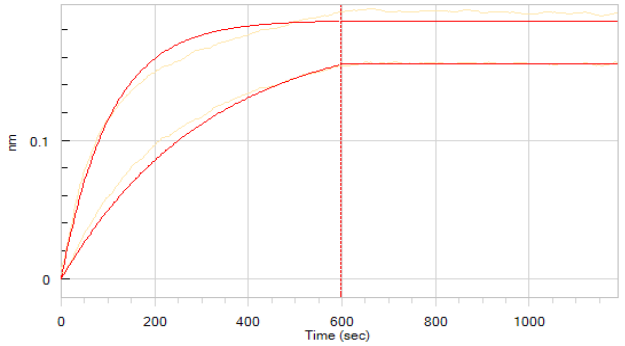
Exhibit ultra-high affinity for spike protein (>100-fold improvement)

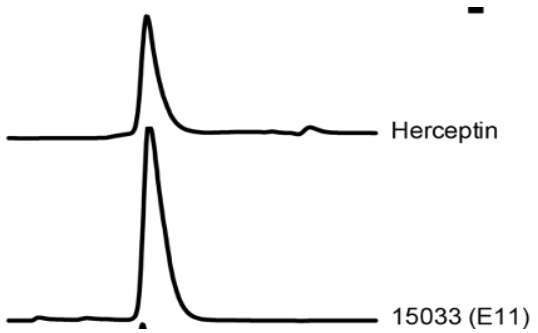
IgG ID	K_D (pM)	k_{on} ($10^5 M^{-1}s^{-1}$)	k_{off} ($10^{-5}s^{-1}$)
parental IgG 15033	320 ± 10	9.6 ± 0.1	31 ± 0.8
hk L3 AM1	170 ± 14	8.5 ± 0.2	15 ± 1.2
hk L3 AM2	610 ± 19	12 ± 0.3	72 ± 1.5
hk L3 AM3	420 ± 17	8.5 ± 0.2	36 ± 1.2
hk L3 AM4	330 ± 10	10 ± 0.1	34 ± 0.8
hk L3 AM5	500 ± 10	11 ± 0.1	5 ± 0.8
hk L3 AM6	76 ± 14	8.4 ± 0.2	6.4 ± 1.2
hk L3 AM7	<1	8.6 ± 0.2	<0.1
hk L3 AM8	77 ± 10	10 ± 0.2	7.8 ± 1.0
hk L3 AM9	360 ± 16	12 ± 0.3	42 ± 1.5
hk L3 AM10	100 ± 10	19 ± 0.4	19 ± 1.3
hk L3 AM11	200 ± 10	12 ± 0.2	24 ± 1.0
hk L3 AM12	51 ± 12	13 ± 0.3	6.4 ± 1.5
hk L3 AM13	210 ± 10	14 ± 0.2	30 ± 1.0
hk L3 AM14	130 ± 10	13 ± 0.2	17 ± 1.1
hk L3 AM15	400 ± 11	14 ± 0.2	54 ± 1.2
hk L3 AM16	340 ± 12	13 ± 0.2	44 ± 1.3
hk L3 AM17	250 ± 10	14 ± 0.2	34 ± 1.1

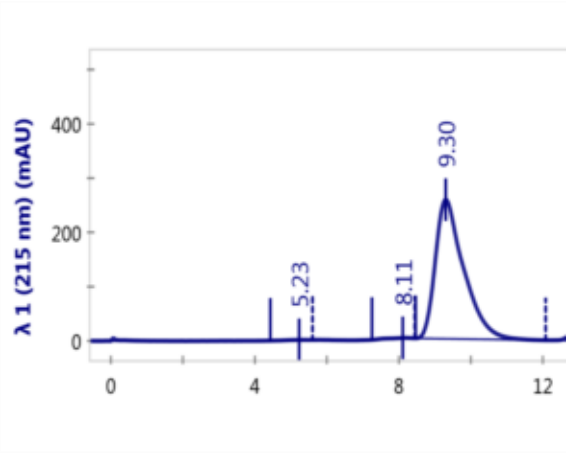
15033 parent (85 mg/L)



15033-AM7 (150 mg/L)







Anti-COVID-19 Antibodies: Beyond the RBD

ViRNA therapeutics

- Selected against purified SARS-CoV-2 RBD
  - Screened 384 clones by ELISA
  - Identified 358 RBD-positive clones
  - 92 sequence-unique, RBD-binding clones

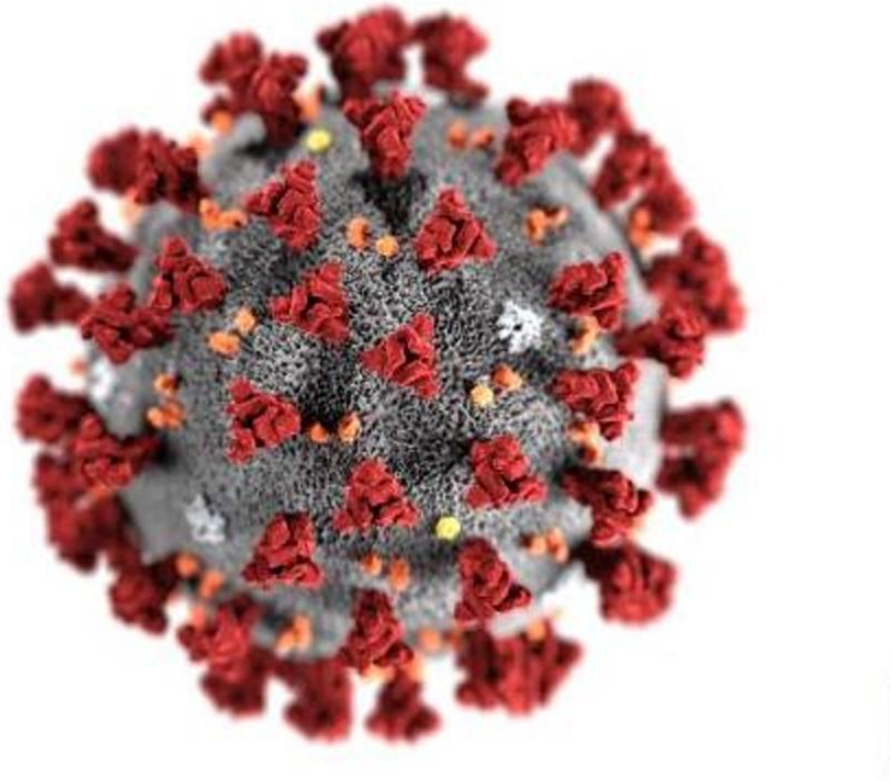
- Selected against VLPs pseudo-typed with SARS-CoV-2 spike
  - Screened 192 clones by ELISA
  - Identified 61 spike-positive clones
  - 24 sequence-unique clones (23 spike-binding, 1 RBD binding)

- Selected against purified SARS-CoV-2 spike protein
  - Screened 192 clones by ELISA
  - Identified 184 spike-positive clones
  - 87 sequence-unique clones

Project Timeline: Anti-COVID19 Antibody Development

Parameter	Method	Numbers	Timeline
Pooled selections	Phage display	$>10^{10}$	3/24 – 3/29
Binding screen	Phage ELISA	384	3/30
Host receptor blocking screen	Phage ELISA	358	3/30
IgG production	Mammalian cell expression	66	3/31 – 4/11
Identification of unique IgGs	DNA sequencing	38	4/8
IgG binding to virus	ELISA	38	4/12
Receptor blocking confirmation	ELISA	~15	4/12
Affinity	Quantitative virus binding	~10 (sub-nanomolar)	4/13
Biophysical characteristics	Yield, solubility, heterogeneity	4 (top leads)	4/13
In vitro virus neutralization	*In vitro* cell assay	4	4/27
In vivo virus neutralization	Human trials	1	9/30

Questions



Answers