COVID-19 Therapeutic Development With Synthetic Antibody Technology

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Natural Antibodies

Antigen injection leads to immunization, which results in the isolation of immune cells. These cells are then used to form antibody-producing cells through the process of clonal expansion. The antibody-forming cells are then fused with tumor cells to create hybridomas. These hybridomas are screened for production of the desired antibody and the antibody-producing hybridomas are cloned.

Monoclonal antibodies are produced through this process.
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
COVID-19 and the Immune System

SARS HCoV-2

Host receptor

Viral infection

Antibody

Antibody-blocked host receptor

Blockade of viral infection
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
Validated Antibody Platform for Anti-Virals

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


- 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
Effectiveness of convalescent plasma therapy in severe COVID-19 patients

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Chest CTs of two patients. (A) Chest CT of patient 9 obtained on February 9 (7 dpo) before CP transfusion showed ground glass opacity with uneven density involving the multiple subsegmental and secondary 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 13 (11 dpo) after CP transfusion (10 mL/kg) showed the absorption of bilateral ground glass opacity after CP transfusion. (C) Chest CT of patient 10 was obtained on February 4 (5 dpo) before CP transfusion (20 mL/kg). The brightness of both lungs was diffusely decreased, and multiple shadows of high density in both lungs were observed. After CP transfusion, patient 10 exhibited high fever and CP showed three 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 COVID-19
- 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

Adverse effects of CP transfusion (most patients did not develop adverse effects common to plasma transfusion, such as allergic reactions, fever, or gushes of blood.)

Discussion

The speed of the inflammatory reaction in COVID-19 patients is very rapid. It is common to observe patients developing ARDS within 2 weeks of infection. After CP transfusion, the inflammatory storm was significantly reduced, heart rate and oxygenation were improved, and lung density became clearer.

Further studies are required to determine the optimal dose, optimal time, and number of CP transfusion to achieve the best clinical outcomes. The definition of primary and secondary outcomes. One dose of 200-300 mL CP transfusion was used to treat severe or critical patients who were initially diagnosed with COVID-19 and were then confirmed by real-time PCR.

Synthetic Neutralizing Antibodies – Proven Stability and Efficacy

-Rapid Development and Scalable Cost-effective Production

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Toronto Recombinant Antibody Centre
A High-throughput synthetic antibody platform applied to COVID-19

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- Minimal targeted synthetic CDR diversity
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Antibodies bind specifically and block ACE2 binding
Antibodies are drug-like

**EC$_{50}$ affinity estimation by ELISA**

$\text{EC}_50 = 0.1 \pm 0.1 \text{ nM}$

**Binding kinetics by BLI**

$K_D \sim 0.6 \text{ nM}$

**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)

<table>
<thead>
<tr>
<th>IgG ID</th>
<th>$K_d$ (pM)</th>
<th>$k_c$ ($10^9 M^{-1}s^{-1}$)</th>
<th>$k_m$ ($10^9 s^{-1}$)</th>
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<tr>
<td>parental IgG 15033</td>
<td>320 ± 10</td>
<td>9.6 ± 0.1</td>
<td>31 ± 0.8</td>
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<tr>
<td>L3AM1</td>
<td>170 ± 14</td>
<td>8.5 ± 0.2</td>
<td>15 ± 1.2</td>
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<tr>
<td>L3AM2</td>
<td>610 ± 19</td>
<td>12 ± 0.3</td>
<td>72 ± 1.5</td>
</tr>
<tr>
<td>L3AM3</td>
<td>420 ± 17</td>
<td>8.5 ± 0.2</td>
<td>36 ± 1.2</td>
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<tr>
<td>L3AM4</td>
<td>330 ± 10</td>
<td>10 ± 0.1</td>
<td>34 ± 0.8</td>
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<td>L3AM5</td>
<td>500 ± 10</td>
<td>11 ± 0.1</td>
<td>5 ± 0.8</td>
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<td>L3AM6</td>
<td>76 ± 14</td>
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<td>6.4 ± 1.2</td>
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<td>L3AM7</td>
<td>&lt;1</td>
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<td>&lt;0.1</td>
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<td>L3AM8</td>
<td>77 ± 10</td>
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<td>L3AM9</td>
<td>360 ± 16</td>
<td>12 ± 0.3</td>
<td>42 ± 1.5</td>
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<td>L3AM10</td>
<td>100 ± 10</td>
<td>19 ± 0.4</td>
<td>19 ± 1.3</td>
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<td>L3AM11</td>
<td>200 ± 10</td>
<td>12 ± 0.2</td>
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<td>L3AM12</td>
<td>51 ± 12</td>
<td>13 ± 0.3</td>
<td>6.4 ± 1.5</td>
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<td>L3AM13</td>
<td>210 ± 10</td>
<td>14 ± 0.2</td>
<td>30 ± 1.0</td>
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<td>L3AM14</td>
<td>130 ± 10</td>
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<td>17 ± 1.1</td>
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<td>L3AM15</td>
<td>400 ± 11</td>
<td>14 ± 0.2</td>
<td>54 ± 1.2</td>
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<td>L3AM16</td>
<td>340 ± 12</td>
<td>13 ± 0.2</td>
<td>44 ± 1.3</td>
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<td>L3AM17</td>
<td>250 ± 10</td>
<td>14 ± 0.2</td>
<td>34 ± 1.1</td>
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</table>

15033 parent (85 mg/L)

15033-AM7 (150 mg/L)
Synthetic antibodies enable modular design: Facile engineering of multivalent drug-like antibodies
Synthetic multivalent antibodies: Enhanced affinity and potency in anti-viral assays
### Project Timeline: Anti-COVID19 Antibody Development

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Numbers</th>
<th>Timeline</th>
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<tbody>
<tr>
<td>Pooled selections</td>
<td>Phage display</td>
<td>$&gt;10^{10}$</td>
<td>3/24 – 3/29</td>
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<tr>
<td>Binding screen</td>
<td>Phage ELISA</td>
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<td>3/30</td>
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<td>Host receptor blocking screen</td>
<td>Phage ELISA</td>
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<td>3/30</td>
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<td>IgG production</td>
<td>Mammalian cell expression</td>
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<td>3/31 – 4/11</td>
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<td>Identification of unique IgGs</td>
<td>DNA sequencing</td>
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<td>4/8</td>
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<tr>
<td>IgG binding to virus</td>
<td>ELISA</td>
<td>38</td>
<td>4/12</td>
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<tr>
<td>Receptor blocking confirmation</td>
<td>ELISA</td>
<td>~15</td>
<td>4/12</td>
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<tr>
<td>Affinity</td>
<td>Quantitative virus binding</td>
<td>~10 (sub-nanomolar)</td>
<td>4/13</td>
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<tr>
<td>Biophysical characteristics</td>
<td>Yield, solubility, heterogeneity</td>
<td>4 (top leads)</td>
<td>4/13</td>
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<td><em>In vitro</em> virus neutralization</td>
<td><em>In vitro</em> cell assay</td>
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<td>4/27</td>
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<tr>
<td><em>In vivo</em> virus neutralization</td>
<td>Human trials</td>
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<td>Soon</td>
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Anti-COVID-19 Antibodies: Beyond the RBD

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

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

• Selected against purified SARS-CoV-2 spike protein
  o Screened 192 clones by ELISA
  o Identified 184 spike-positive clones
  o 87 sequence-unique clones
Lung-on-a-chip: Human Relevant System for Testing COVID-19 Therapeutics

- Extracellular matrix and cell interactions
- Cell shape and cyto-architecture
- Tissue-tissue interactions
- Mechanical forces
- Dynamic flow system
- Resident or circulating immune cells can be included

Thousands of potential biologic drugs: Well-suited for testing in advanced cell/tissue models

Nicole Kleinstreuer