July 2020 Update: Vaccines targeting COVID-19

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• Vaccine Development Path (Overview)
• Development of COVID-19 vaccines
• Current highlights of leading candidates
• Updates from the Lehrer Laboratory (UH)
Vaccine Development Path

Conventional: inactivated and attenuated pathogens

Subunit vaccines (conventional or recombinant)

Recombinant approaches: DNA, RNA, viral vector for antigen delivery

## Manufacturing Processes

### Conventional vaccines

<table>
<thead>
<tr>
<th>Manufacturing Process</th>
<th>Vaccines Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated virus</td>
<td>Smallpox, polio, measles, mumps, rubella, varicella, rotavirus, herpes zoster, influenza, yellow fever</td>
</tr>
<tr>
<td>Inactivated purified virus</td>
<td>Polio, Japanese encephalitis, hepatitis A, influenza, rabies</td>
</tr>
<tr>
<td>Live attenuated bacterium</td>
<td>Tuberculosis, typhoid</td>
</tr>
<tr>
<td>Whole-cell inactivated bacteria</td>
<td>Whole-cell pertussis</td>
</tr>
<tr>
<td>Purified protein</td>
<td>Acellular pertussis</td>
</tr>
<tr>
<td>Purified protein toxoid</td>
<td>Tetanus, anthrax, diphtheria</td>
</tr>
</tbody>
</table>

### Next-generation approaches

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified virus-like particles (VLP)</td>
<td>Hepatitis B, human papilloma¹</td>
</tr>
<tr>
<td>Purified polysaccharide</td>
<td>Pneumococcal, typhoid</td>
</tr>
<tr>
<td>Polysaccharide conjugated to carrier proteins</td>
<td>Pneumococcal, <em>H. influenzae</em> type b, <em>N meningitidis</em></td>
</tr>
<tr>
<td>Plasmid DNA</td>
<td>GTU®-encoded protein HIV vaccine candidate</td>
</tr>
<tr>
<td>Adenovirus DNA delivery</td>
<td>HIV vaccine candidate</td>
</tr>
</tbody>
</table>

¹VLP reassembled from type 16 and 18 type specific L1 proteins expressed and purified from insect cells infected with a recombinant baculovirus or HPV 6, 11, 16 and 18 type specific L1 proteins expressed and purified from yeast containing L1 expression plasmids.
Immune mechanisms associated with different vaccination strategies differ in their extent of cell-mediated and humoral immunity.

From: Barrett, AD (2008). Nature Biotechnology 26, 525-526
New vaccine development can take up to 20 years, and typically costs between US $500 million and $1 billion, including construction of facilities for manufacture.\textsuperscript{1,2}

FDA - Regulatory Process

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Coronaviruses: Risk of ADE?

Effect appears to be linked to quality of immune response
**Spike protein function:**
- Receptor binding
- Membrane fusion

Class I fusion glycoprotein on the surface of the virus responsible for gaining entry into host cells.

The S protein is a trimeric protein that exists in a metastable prefusion state.

~180 kDa if fully glycosylated, 22 glycosylation sites.

The monomer consists of S1 and S2 subunits that are associated non-covalently.

S1 subunits forming an interwoven cap that rests atop the spring-loaded S2 stem.

Conformation that undergoes a substantial structural rearrangement to fuse the viral membrane with the host cell membrane.

Pallesen et al. PNAS 2017

Wrapp and Wang et al. Science 2020
S1 subunit contains the RBD and responsible for receptor recognition

S2 subunit responsible for membrane fusion

The SARS-CoV 2 S shares 76% aa homology with SARS-CoV

Ou et al. Nat Comm 2020

S1 homology is about 64%
RBD homology is about 74%

Ou et al. Nat Comm 2020

RBD domain exhibits hinge like motion switches between several open and one closed formation

Receptor (Ab) binding is only possible when RBD is in open position

Wrapp and Wang et al. Science 2020
Cyro-EM Structure of SARS-CoV-2 glycoprotein – receptor binding

**Binding of RBD to receptor induces shedding of S1 and cleavage of S2 cleavage site**

- Results in conformational change in S2 to highly stable post-fusion state – potential need to stabilize pre-fusion conformation
SARS CoV-2 Spike binds to ACE2

Negative-stain EM

Surface Plasmon Resonance

Biolayer Interferometry

~10-20-fold difference

Wrapp and Wang et al. Science 2020

Biolayer Interferometry

~ Higher affinity of SARS CoV 2 to ACE2

Wrapp and Wang et al. Science 2020

ACE2 binding to SARS-CoV 2 and SARS-CoV S

Biolayer Interferometry

K_D: 1.2 nM
K_on: 1.4x10^5 M^{-1}s^{-1}
K_off: 1.6x10^{-4} s^{-1}

K_D: 5 nM
K_on: 1.4x10^5 M^{-1}s^{-1}
K_off: 7.1x10^{-4} s^{-1}

Walls et al. Cell 2020
Journal of Clinical and Experimental Hepatology DOI:
(10.1016/j.jceh.2020.06.003)
Overview - COVID-19 Vaccines

- Total number of (known) Vaccine Candidates under development: 199 (20: unknown platform used)
- **Conventional:** 13 (inactivated), 4 (live-attenuated)
- **Virally vectored vaccines:** 23 (non-rep), 18 (rep)
- **Genetic vaccines:** 16 (DNA), 27 (RNA)
- **Recombinant subunits:** 63 (protein), 15 (VLP)
- **Passive Immunization** - convalescent serum and antibody therapies: 44

Source: Milken Institute COVID-19 tracker
Accessed 07/29/2020
# COVID-19 Vaccines – Clinical Status

<table>
<thead>
<tr>
<th>Platform</th>
<th>Pre-clinical</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated</td>
<td>9</td>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>live attenuated</td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td>protein subunits</td>
<td>59</td>
<td>4</td>
<td></td>
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<tr>
<td>VLP</td>
<td>14</td>
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</tr>
<tr>
<td>DNA</td>
<td>13</td>
<td>3</td>
<td></td>
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</tr>
<tr>
<td>RNA</td>
<td>23</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>non-replicating viral vector</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>replicating viral vector</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
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Source: Milken Institute COVID-19 tracker
Accessed 07/29/2020
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Moderna – RNA vaccine candidate

Corbett et al. 2020, bioRxiv preprint doi:
https://doi.org/10.1101/2020.06.11.145920.
RNA: Mouse Immunogenicity

Continued with 100µg dose level
mRNA: Antibody and Neutralization Responses

Antibody Responses in Rhesus Macaques (mRNA)

DOI: 10.1056/NEJMo2024671
T-Cell Responses after mRNA-1273 Vaccination

A Th1 Responses

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>10 µg</th>
<th>100 µg</th>
<th>mRNA-1273 Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>0/7</td>
<td>4/8</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P=0.002</td>
<td>P=0.007</td>
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</table>

B Th2 Responses

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>10 µg</th>
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<th>mRNA-1273 Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>0/7</td>
<td>0/8</td>
<td>2/7</td>
<td></td>
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</table>

C CD40L

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>10 µg</th>
<th>100 µg</th>
<th>mRNA-1273 Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>0/7</td>
<td>3/8</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=0.003</td>
<td></td>
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<td></td>
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</table>

D Tfh Interleukin-21

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>10 µg</th>
<th>100 µg</th>
<th>mRNA-1273 Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>0/7</td>
<td>4/8</td>
<td>7/7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P=0.009</td>
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</tbody>
</table>

Efficacy: Upper/Lower Respiratory Viral Load (mRNA)

A Subgenomic RNA in BAL Fluid
- PBS
- mRNA-1273, 10 µg
- mRNA-1273, 100 µg

B Subgenomic RNA in Nasal Swabs
- PBS
- mRNA-1273, 10 µg
- mRNA-1273, 100 µg

DOI: 10.1056/NEJMo2024671
Summary: mRNA vaccine

- Good immunogenicity after two doses in mice, NHP’s and humans
- Balanced responses show that delivery seems efficient for this candidate
- Potent virus neutralization shown
- Reduction in viral loads in Rhesus model

- Durability of immunity?
- Adverse reactions and exclusion criteria?
- Efficacy after only one dose and kinetics of immune response?
Ad5-vectored platform (CanSinobiologics)

Specific antibody responses to RBD, neutralising antibodies to live severe acute respiratory syndrome coronavirus 2 and pseudovirus post vaccination
https://doi.org/10.1016/S0140-6736(20)31605-6

➤ Single (high) dose – problem: pre-existing immunity to vector
ChAdOx Platform – Human clinical testing

Commercial Developer: AstraZeneca

Folegatti et al. 2020. The Lancet DOI: (10.1016/S0140-6736(20)31604-4)
ChAdOx Platform – Human clinical testing

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The Lancet DOI: (10.1016/S0140-6736(20)31604-4)
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Study used model antigen – S1 subunit from mammalian cells (SinoBiological)
COVID-19 Vaccine – Adjuvant

Study used model antigen – S1 subunit from mammalian cells (SinoBiological)

Virus neutralizing antibody titers against wild-type SARS-CoV-2

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Adjuvant</th>
<th>PRNT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>PRNT&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2 spike S1</td>
<td>CoVaccine HT</td>
<td>1620</td>
<td>1620</td>
</tr>
<tr>
<td>SARS-CoV-2 spike S1</td>
<td>Alhydrogel</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>SARS-CoV-2 spike S1</td>
<td>None</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>None</td>
<td>CoVaccine HT</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>
Detection of IFN-γ secreting cells from mice immunized with SARS-CoV-2 vaccines

- Potent humoral immune responses with good virus-specificity
- With lead adjuvant high virus neutralizing antibody titers
- Potent induction of Th1-type immunity including IFN-γ secretion from peptide-stimulated splenocytes

Study used model antigen – S1 subunit from mammalian cells (SinoBiological)
Further Development

- Testing thermostabilization methods for selected antigen (in collaboration with Soligenix)
- Determine optimal antigen and adjuvant dosage
- Develop fully scaleable production method for selected Spike antigen (in collaboration with Hawaii Biotech)
- Finalize thermostabilization method development for a trivalent formulation including adjuvant (in collaboration with Soligenix)
- Define correlates of protection for SARS-CoV-2 vaccine using mouse and non-human primate studies (in collaboration with BIOQUAL, Inc.)
- Manufacture clinical grade vaccine and test safety and efficacy in human clinical trials
Acknowledgements

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Jake Yalley
Laurent Pessaint

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