Learning from SARS-CoV-2 (COVID-19): Emergence, Disease, Biology, and Antivirals

Denison Lab 2019

Lab Alumni and new

Jordan Anderson-Daniels
Amelia George
Nicole Sexton
Learning from SARS-CoV-2 (COVID-19): Emergence, Disease, Biology, and Antivirals

Denison Lab 2019

Lab Alumni and new

Jordan Anderson-Daniels
Amelia George
Nicole Sexton
Thanks

**UNC- Chapel Hill**
Ralph Baric
Amy Sims
Tim Sheahan
Rachel Graham

**Gilead Sciences**
Joy Y. Feng
Danielle Porter
Thomas Cihlar

**Emory University**
George Painter
Mike Natchus
Greg Bleumling

**Funding**
U19-AI109680.
R01 AI132178
R01 AI108197
Vaccine Research Center- NIH
Goals

• FAQs, Fakes, and Facts
• SARS-CoV-2 infection (COVID-19)
• Human, zoonotic, and potential pandemic Coronaviruses
• Replication and Antiviral Strategies
• Plenty of Time for Questions
FAQs, Fakes, and Facts

• What about pediatric disease?
• Influenza (the flu) causes more illness and death so why are we worrying about this!
• The mortality rate is low - what is the big deal?
• Can the virus spread asymptptomatically?
• How is it transmitted?
• Why is it so hard to control?
FAQs, Fakes, and Facts

- They say “the coronavirus – is there only one?"
- Can I get the virus from packages from China -?
- Was the cruise ship quarantine a good idea?
- Why did SARS disappear and this virus is pandemic?
- How long will a vaccine take?
- Do we have any drugs to treat this?
FAQs, Fakes, and Facts

- Immunity to other CoVs is protective, irrelevant, detrimental
- Anti-HIV and anti-Flu antivirals work against the coronavirus
- The virus is mutating and that is why it is spreading
- The virus is man-made and engineered!
- Then how did it get into humans?
- The virus will go away when the weather warms up!
- The virus will become a seasonal respiratory virus like rhinoviruses or endemic coronaviruses!
Human Endemic Coronaviruses (HCoVs)

- 229E, HKU1, NL63, OC43
- Up to 15-30% of human colds
- No durable immunity – frequent cycles of infection
- Upper Respiratory Infections – most common
- Lower Respiratory Infections – immunocompromised patients - severity and contributions still undefined
- No vaccines or antivirals licensed or in use
Human Endemic Coronaviruses (HCoVs)

- 229E, HKU1, NL63, OC43
- Up to 15-30% of human colds
- No durable immunity – frequent cycles of infection
- Upper Respiratory Infections – most common
- Lower Respiratory Infections – immunocompromised patients - severity and contributions still undefined
- No vaccines or antivirals licensed or in use
Emerging Coronaviruses

- **SARS-CoV (2002-2004). – Severe Acute Respiratory Syndrome**
  - >8000 cases, 10% mortality, 32 countries in 3 months.
  - Bats – Civet Cats / Raccoon Dogs / Humans

- **MERS-CoV (2012-Present) (Middle East Respiratory Syndrome)**
  - > 2500 cases, ~35% mortality, 27 countries
  - Bats – Camels – Humans

- **nCoV-19, COVID-19, SARS-CoV-2 (2019-present)**
  - > >75,000 cases, >2% mortality?
  - >29 countries
2018 U19 Application : Significance and Impact

“The epidemics SARS- and MERS-CoVs in humans has confirmed the capacity for CoV to emerge from animal reservoirs to cause severe human disease”

“SARS-like bat CoVs are currently circulating in nature that utilize the human SARS-CoV receptor, replicate efficiently in primary human airway cells, and are resistant to existing pre-clinical therapeutic antibodies and vaccines”

“With increasing overlap of human and wild animal ecologies, the potential for future severe zoonotic CoV emergence is high”
Accelerating Emergence of Zoonotic CoVs with Pandemic Potential
Models for CoV Zoonoses

Host Range Mutations

"Ready-Made" Pre-Pandemic

Hurdles to Virus Movement and Adaptation

Adapted from M.Vignuzzi

Virulence, Morbidity, Mortality

Escape mutants - decoys

Adaptive immunity

Tissue barriers

Pathology

Innate immunity

Local and systemic replication

Specialized mutants

Naive host population

Environmental stability
Phylogeny of SARS-like betacoronaviruses including novel coronavirus SARS-CoV-2

Showing 45 of 45 genomes.

Feb 24, 2020

https://nextstrain.org/groups/bla/beta-cov

New Clade of SARS-like Viruses
5000 nt difference

SARS-CoV-2

SARS-CoV

SARS-Like-CoV

Pangolin

Wuhan SARS-CoV-2

BAT RaTG13 from Cave Bat in Yunnan Province
96% nt identity (1200 nt)
SARS-COV-2 Incubation Period

Average 5 day incubation period
Range 2-14 days

Guan NEJM 2020
<table>
<thead>
<tr>
<th>Age Range</th>
<th>Cases N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9 years</td>
<td>416 (0.9)</td>
</tr>
<tr>
<td>10-19 years</td>
<td>549 (1.2)</td>
</tr>
<tr>
<td>20-29 years</td>
<td>3,619 (8.1)</td>
</tr>
<tr>
<td>30-39 years</td>
<td>7,600 (17.0)</td>
</tr>
<tr>
<td>40-49 years</td>
<td>8,571 (19.2)</td>
</tr>
<tr>
<td>50-59 years</td>
<td>10,008 (22.4)</td>
</tr>
<tr>
<td>60-69 years</td>
<td>8,583 (19.2)</td>
</tr>
<tr>
<td>70-79 years</td>
<td>3,918 (8.8)</td>
</tr>
<tr>
<td>≧80 years</td>
<td>1,408 (3.2)</td>
</tr>
</tbody>
</table>
COVID-19 Clinical Symptoms

- Fever (83-98%)
- Cough (46-82%)
- Myalgia or fatigue (11-44%)
- Shortness of breath (31%)
- Less common symptoms: diarrhea, productive sputum
- Potential for worsening clinical course during second week of symptoms
- ARDS developed in 17-29% of hospitalized patients
- Secondary infection in 10% of hospitalized patients

From: Annabelle de St Maurice
COVID-19 Disease Severity

• 36,160 cases (81%) reported mild symptoms
• 6,168 cases (13.8%) reported severe symptoms
• 2,087 cases (4.7%) were critically ill
• Case fatality higher among those with comorbid conditions (2-12%) compared to those with no comorbidities (0.9%)

From: Annabelle de St Maurice

China CDC Weekly Report February 17, 2020
### Mortality of Confirmed Patients in China 2/11/20

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cases N (%)</th>
<th>Deaths N (%)</th>
<th>Case Fatality Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>44,672</td>
<td>1,023</td>
<td>2.3%</td>
</tr>
<tr>
<td>0-9 years</td>
<td>416 (0.9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10-19 years</td>
<td>549 (1.2)</td>
<td>1 (0.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>20-29 years</td>
<td>3,619 (8.1)</td>
<td>7 (0.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>30-39 years</td>
<td>7,600 (17.0)</td>
<td>18 (1.8)</td>
<td>0.2</td>
</tr>
<tr>
<td>40-49 years</td>
<td>8,571 (19.2)</td>
<td>38 (3.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>50-59 years</td>
<td>10,008 (22.4)</td>
<td>130 (12.7)</td>
<td>1.3</td>
</tr>
<tr>
<td>60-69 years</td>
<td>8,583 (19.2)</td>
<td>309 (30.2)</td>
<td>3.6</td>
</tr>
<tr>
<td>70-79 years</td>
<td>3,918 (8.8)</td>
<td>312 (30.5)</td>
<td>8.0</td>
</tr>
<tr>
<td>≥80 years</td>
<td>1,408 (3.2)</td>
<td>208 (20.3)</td>
<td>14.8</td>
</tr>
</tbody>
</table>

From: Annabelle de St Maurice

China CDC Weekly Report February 17, 2020
SARS-COV-2 Transmission

• Based on knowledge of other coronaviruses (SARS and MERS)

• Person to person via respiratory droplets among close contacts
  • Within 6 feet of a patient with SARS-COV-2 for a prolonged period of time
  • Having direct contact with infectious secretions from a patient with SARS-COV-2 (sputum, serum, blood, respiratory droplets)
  • SARS-COV-2 has been detected in stool but clinical significance is unknown

• Routine environmental cleaning and disinfection is appropriate
Need for Antivirals against CoVs

- Broad diversity of CoVs in bats with demonstrated capability to infect human cells animal models – “outbreak ready”
- Failure of antibodies to neutralize “future” zoonotic CoVs and loss of cross protection by vaccines
- Time to develop vaccines differs from trajectory of epidemic
- Universal vaccines across all CoV PPP groups will be difficult and potentially with gaps or not possible
- Potential for “off the shelf” use toward highly conserved functions
Goals for CoV antiviral development

• Broadly active against diverse coronaviruses

• High barrier to resistance - limited genetic paths, high fitness cost (loss of virulence)

• Extended therapeutic window for prevention, amelioration, treatment,

• Additional: decrease transmission: oral administration
Coronavirus Countermeasures

Direct acting antivirals (DAA’s): treatment; prophylaxis; transmission

Monoclonal antibodies: treat; “passive immunization” to prevent

Host Directed therapy: modify or block disease – extend therapeutic window
Coronavirus Replication

- Obligate Intracellular Replication
- Virus binding – ACE2
- RNA Genome uncoating
- Genome TRANSLATION and PROTEIN PROCESSING
- Replicase assembly and genome replication
- Virus assembly
- Virus egress - release

Coronavirus Replication

- Subgenomic (−)RNAs
- Subgenomic (+)mRNAs
- Essential functions and viral components:
  - Entry - Spike
  - Translation
  - Proteolysis - nsp3 and nsp5
  - Replication and Transcription - (nsp7-nsp14)
  - Assembly and Release - structural proteins


Spike protein

- Host cell receptor binding and virus entry
- Determinant of species specificity, tropism and immunity
- Problem of lack of cross protection

Spike protein

Spike
Coronavirus Replication

Monoclonal antibodies – Crowe lab and others

Vaccines – Moderna Vaccine – RNA only
- Designed and implemented at NIH-VRC: Barney Graham
- Expected Phase 1 in humans by April 2020
- Testing for antibody human antibody response in Denison lab via VTEU (Creech)

Coronavirus Replication

- Genome RNA translated to polyproteins
- Polyproteins processed by viral proteases: PLP and 3CLpro
- Blocking protease activity aborts virus replication
Coronavirus Replication

- Genome RNA is translated to polyproteins of all replicase proteins
- Polyproteins are processed by viral proteases: PLP and 3CLpro
- Blocking protease activity aborts virus replication

Protease inhibitors as Coronavirus antivirals

CoV proteases PLP, 3CLpro
Coronavirus Replication

Virus RNA synthesis

- Replicase Proteins assemble and modify host membranes into replication factories
- RdRp- polymerase as critical protein
- Highly conserved across coronaviruses

Coronaviruses assemble a multiprotein replicase complex
Viral RNA-Polymerases are structurally conserved and known targets for antivirals

Nucleoside and base analogs are efficacious antivirals against multiple RNA and DNA virus polymerases

- **Ribavirin**: HCV, RSV
- **Azidothymidine (AZT)**: HIV
- **Sofosbuvir**: HCV
- **Lamivudine (3TC)**: HIV, HBV
- **Acyclovir**: Herpes viruses
- **Favipiravir**: Influenza
Problem: Coronaviruses are resistant to many nucleoside analog inhibitors

MOI = 0.01 PFU/cell
24 h p.i.

Smith et al. PLOS Path. 2013.
Problem: Coronaviruses are resistant to many nucleoside analog inhibitors
• Coronaviruses encode **Proofreading ExoN**
• Removes mis-incorporated nucleotides
• Confers high fidelity replication (up to 20-fold)
Inactivate Exonuclease Proofreading
Proofreading nsp14-ExoN is responsible for CoV native resistance to nucleoside analogs

Adapted from Smith et al. PLOS Path. 2013.
Question:

Can nucleoside analogs inhibit CoVs in setting of proofreading exonuclease?
Remdesivir (RDV)

**NIH U19 support**

- **Fall 2014** AD3C team demonstrates in vitro anti-CoV activity of RDV
- **March 2018** Agostini et al. RDV mechanism of action
- **June 2017** Sheahan, et al. Broad-spectrum efficacy of RDV against epidemic and zoonotic CoV

**NIH-R01 support**

- **Aug 2018.** RDV enters randomized controlled trial of Ebola virus disease therapeutics
- **Sept 2019** Brown et al. Broad-spectrum efficacy of RDV against human endemic and zoonotic Δ-CoVs
- **Jan 2020** Sheahan et al. Superior efficacy of RDV over standard of care for MERS-CoV
- **Jan 25 2020** First US case of 2019-nCoV treated with RDV
- **Feb 3 2020** RDV China phase III trial to fight coronavirus
Remdesivir and β-D-\(^{\text{N}^4}\)-Hydroxycytidine (EIDD-1931/2801, NHC) inhibit CoV replication

Virus Titer Reduction (VTR)
6 logs (million x)

EIDD-1931 / NHC Emory
VTR 5 logs (100,000x)

Agostini et al mBio 2017
Agostini et al J Virol 2019
Remdesivir and β-D-\(N^A\)-Hydroxycytidine (EIDD-1931/2801, NHC) inhibit CoV replication.

\[ EC_{50} = 0.03 \, \mu M \]
\[ EC_{50} = 0.17 \, \mu M \]

Remdesivir (Gilead)

EIDD-1931 / NHC Emory

Change in Viral Titer (Log_{10} PFU/mL)

VTR 6 logs

VTR 5 logs

\[ \text{EC}_{50} = 0.03 \, \mu M \]

\[ \text{EC}_{50} = 0.17 \, \mu M \]

Agostini et al mBio 2017

Agostini et al J Virol 2019
Remdesivir inhibits human CoVs and potential zoonotic Bat-CoVs

Pre-Pandemic Bat CoVs

<table>
<thead>
<tr>
<th>α-CoV</th>
<th>β–2c MERS-like</th>
<th>β–2b SARS-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCoV-NL63</td>
<td>Bat-CoV HKU5</td>
<td>Bat-CoV SCH014</td>
</tr>
<tr>
<td></td>
<td>Bat-CoV HKU3</td>
<td>Bat-CoV WIV1</td>
</tr>
</tbody>
</table>

MOI = 0.5 PFU/cell
48 h p.i.

Murine Hepatitis Virus (MHV)

MERS-CoV

SARS-CoV

HCoV-HKU1

HCoV-OC43

HCoV-229E

HCoV-NL63

2019-nCoV

“SARS-like”

Bt-SHC014

Bt-WIV1

HCoV-HKU5

Inhibited by Remdesivir
Remdesivir inhibits virus RNA synthesis by chain termination – with decrease in virus titer

![Graph showing change in viral titer and change in RNA genome with remdesivir concentration.]

MOI = 1 PFU/cell
10 h p.i.

Adapted from Agostini et al. *mBio*. 2018.
Role of proofreading in remdesivir inhibition

MOI = 0.01 PFU/cell
24 h p.i.

WT EC$_{50}$ = 0.087 μM
ExoN(-) EC$_{50}$ = 0.019 μM

Adapted from Agostini et al. _mBio_. 2018.
Remdesivir (GS-5734) given before or 1-day post-exposure decreases virus and disease in lethal SARS-CoV mouse infection

IV administration before or early after infection
• Decreases virus titer
• Decreases weight loss
• Decreases lung pathology
• Improves lung function

*Given later in infection
• Decreases virus titer but not lung disease or mortality

Sheahan, Sims et al. Sci Trans Medicine 2017
Questions:

• *Does Remdesivir select for resistance*

• *What is consequence of resistance*
Two mutations selected in the RdRp after 23 passages in the presence of Remdesivir

- F476L
- V553L

Reduction in Virus Titer

- [remdesivir]
- WT

Less sensitive

6 fold resistance
Remdesivir resistance mutations are less fit than WT in vitro and attenuated in vivo

MOI = 0.01 PFU/cell
20 h.p.i

Adapted from Agostini et al. *mBio*. 2018.
Remdesivir Resistance – difficult and detrimental

- Resistance very difficult to select
- Resistance results in loss of fitness (weaker non-competitive virus)
- Resistance results in attenuation (loss of virulence in mice)
Remdesivir - IV

- Potently inhibits multiple divergent CoVs
- Resistance has high barrier and detrimental to virus
- Efficacious for prophylaxis in mouse model of lethal SARS-CoV
- Decreases disease and virus titer when administered early in infection
- Active against SARS-CoV-2 in vitro

(Andrea Pruijssers)
Coronavirus Countermeasures

Direct acting antivirals (DAA’s): treatment; prophylaxis; transmission

Monoclonal antibodies: treat; “passive immunization” to prevent

Host Directed therapy: modify or block disease – extend therapeutic window

COMBINATIONS
FAQs, Fakes, and Facts

• What about pediatric disease?
• Influenza (the flu) causes more illness and death so why are we worrying about this!
• The mortality rate is low - what is the big deal?
• Can the virus spread asymptotically?
• How is it transmitted?
• Why is it so hard to control?
Don’t worry about this flooding, it happens every year and what can you do?

The Flu-ood
Coronavirus

Yeah I see it. But don’t worry about it, because this flood is REALLY BAD!

The Flu-ood
FAQs, Fakes, and Facts

• *What about pediatric disease?*

• *Influenza (the flu) causes more illness and death so why are we worrying about this!*

• *The mortality rate is low - what is the big deal?*

• *Can the virus spread asymptomatically?*

• *How is it transmitted?*

• *Why is it so hard to control?*
FAQs, Fakes, and Facts

• They say “the coronavirus – is there only one?
• Can I get the virus from packages from China -?
• Was the cruise ship quarantine a good idea?
• Why did SARS disappear and this virus is pandemic?
• How long will a vaccine take?
• Do we have any drugs to treat this?
FAQs, Fakes, and Facts

- Immunity to other CoVs is protective, irrelevant, detrimental
- Anti-HIV and anti-Flu antivirals work against the coronavirus
- The virus is mutating and that is why it is spreading
- The virus is man-made and engineered!
- Then how did it get into humans?
- The virus will go away when the weather warms up!
- The virus will become a seasonal respiratory virus like rhinoviruses or endemic coronaviruses!
Learning from SARS-CoV-2 (COVID-19): Emergence, Disease, Biology, and Antivirals

Denison Lab 2019

Lab Alumni and new

Jordan Anderson-Daniels
Amelia George
Nicole Sexton

Maria Agostini
Jim Chappell
Jennifer Gribble
Andrea Pruijssers
Laura Stevens
Tia Hughes
Mark Denison
Xiaotao Lu

Kevin Graepel
Clint Smith
Erica Andres
Brett Case