CMX521: A NUCLEOSIDE WITH PAN-GENOTYPIC ACTIVITY AGAINST NOROVIRUS

Randall Lanier, PhD
Norovirus Infection Is Prevalent and Costly

- Worldwide: ~700 million cases of norovirus each year (~20 million in U.S.)
  - ~219,000 deaths per year\(^1\)

- Economic toll of norovirus is >$60 Billion per year\(^2\)
  - $4.2B in direct health system costs; ≈56B in productivity losses
  - >60% of outbreaks in US occur in long-term care facilities

- Nothing approved for prevention or treatment
  - Norovirus genetic diversity is a significant hurdle for antivirals and vaccines
  - Ideal therapy should have “pan-genotype” activity, i.e., it should work against all strains

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1 PLoS Med 13(4):e1001999
2 PLoS ONE 11(4):e0151219
Norovirus Therapeutics Need to Be Effective Against Diverse Genogroups/Types/Strains

- The nucleotide binding site of the norovirus RNA polymerase is a region that is very similar across diverse noroviruses.
- Chimerix has a unique library of ribonucleosides that are good candidates to bind this region and inhibit viral replication.
The Norovirus RdRp Active Site Is Virtually Identical Across 760 Mouse and Human Noroviruses (GenBank; % identity)

<table>
<thead>
<tr>
<th>RdRp Codon</th>
<th>1422</th>
<th>1423</th>
<th>1424</th>
<th>1425</th>
<th>1426</th>
<th>1427</th>
<th>1480</th>
<th>1481</th>
<th>1521</th>
<th>1522</th>
<th>1523</th>
<th>1524</th>
<th>1571</th>
<th>1572</th>
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</thead>
<tbody>
<tr>
<td>Amino Acid</td>
<td>D</td>
<td>Y</td>
<td>T/S</td>
<td>R/A</td>
<td>W</td>
<td>D</td>
<td>S</td>
<td>G</td>
<td>Y</td>
<td>G</td>
<td>D</td>
<td>D</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>GV (MNV, N=64)</td>
<td>100</td>
<td>100</td>
<td>72/28</td>
<td>99/R</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>GII (HuNoV, N=656)</td>
<td>100</td>
<td>100</td>
<td>100/S</td>
<td>100/R</td>
<td>100</td>
<td>100</td>
<td>99.8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>GI (HuNoV, N=40)</td>
<td>100</td>
<td>100</td>
<td>95/T</td>
<td>100/A</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
The Tale of CMX521 Discovery

"Muna"  
The Cat

"Nora"  
The Mouse

The Poop

The Virus

The Assay

The Library

CMX521

H₂N

O

NH₂

H₂N

O

HO

HO
CMX521 Appears to Have Pan-Genotype Activity against Noroviruses (Calicivirus Family)

<table>
<thead>
<tr>
<th>Virus</th>
<th>MNV-DS2</th>
<th>MNV-CW3</th>
<th>MNV-CR3</th>
<th>HuNoV G1.1</th>
<th>HuNoV GII.3</th>
<th>HuNoV GII.4</th>
<th>HuNoV GII.6</th>
<th>Porcine Sapovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50 (µM)</td>
<td>1.9</td>
<td>0.14</td>
<td>0.26</td>
<td>0.8</td>
<td>&lt;5*</td>
<td>&lt;5*</td>
<td>4.1</td>
<td>&lt;5*</td>
</tr>
<tr>
<td>Cells</td>
<td>RAW</td>
<td>RAW</td>
<td>RAW</td>
<td>HG23</td>
<td>Enteroids</td>
<td>BJAB</td>
<td>BJAB</td>
<td>llc-pk</td>
</tr>
<tr>
<td>Assay</td>
<td>CPE</td>
<td>CPE</td>
<td>CPE</td>
<td>qPCR</td>
<td>qPCR</td>
<td>qPCR</td>
<td>qPCR</td>
<td>qPCR</td>
</tr>
</tbody>
</table>

No/very weak activity against viruses listed below:

- **DNA viruses:** AdV, BKV, EBV, HSV-2, HCMV, HHV6B, HHV8, MCMV, VACV, VZV
- **RNA viruses:** DENV-2, EEEV, ENTV-71, Flu-H1N1, HCV, MEV, POV-3, RSV, RFV, SARS, WNV, YFV

*Concentrations <5 uM being tested*
Mechanism of Action: CMX521-TP Inhibits Norovirus RdRp (Polymerase) Activity by Competing with GTP

<table>
<thead>
<tr>
<th>Nucleotide concentration</th>
<th>CMX521-TP IC\textsubscript{50} (\textmu M)</th>
<th>2’CmeC-TP IC\textsubscript{50} (\textmu M)</th>
<th>Fold Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 \textmu M NTPs</td>
<td>1.38 ± 0.06</td>
<td>1.69 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>0.1 \textmu M NTPs + 200 \textmu M ATP</td>
<td>1.57 ± 0.09</td>
<td>1.68 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>0.1 \textmu M NTPs + 200 \textmu M CTP</td>
<td>1.34 ± 0.06</td>
<td>5.89 ± 0.95</td>
<td>3.5</td>
</tr>
<tr>
<td>0.1 \textmu M NTPs + 200 \textmu M GTP</td>
<td>\textbf{6.5 ± 0.45}</td>
<td>1.7 ± 0.05</td>
<td>\textbf{4.7}</td>
</tr>
<tr>
<td>0.1 \textmu M NTPs + 200 \textmu M UTP</td>
<td>1.31 ± 0.07</td>
<td>1.75 ± 0.10</td>
<td>-</td>
</tr>
</tbody>
</table>

This work conducted in the laboratory of Dr Brent Korba using GII.4 RdRp; data shown are mean +/- SD
Effective Concentrations of the Active Antiviral (CMX521-Triphosphate[TP]) Are Formed Quickly and Degrade Slowly

Caco-2 cells (human colon) treated for 2 hours with 100µM CMX521

drug removed/cells washed

samples taken over next 4 days

- Effective concentrations of active antiviral reached at 2 hours in vitro
- Intracellular half-life of active antiviral is ≈ 24 hours
Oral CMX521 Preferentially Delivers Drug to Gut in Rats

4 hours after Oral administration of 50 mg/kg $[^{14}\text{C}]$CMX521

Radioactive drug preferentially contained in intestines after oral dosing
Norovirus Infects the Top Layer (Epithelia) of the Gut in Humans

- Human norovirus replication limited to gut (primarily at least)
- Primary target cells for a norovirus therapeutic in the gut epithelia
CMX521 Preferentially Delivered to Target Cells with Oral Dosing?

Norovirus (■) infects the epithelial cells of the gut (■ ■ ■)

- More drug in target sites should increase efficacy
- Less drug in non-target sites should also improve safety
Significant Reduction of Norovirus in Mice with Oral CMX521

- Mice received BID CMX521 for two days prior to infection
  - 150 mg/kg orally, 50 mg/kg IP, or both
- Mice orally infected with mouse NV (CR3)
- CMX521 dosing continued for 3 days p.i.
- Feces and tissues collected on day 3 p.i.; norovirus titers determined

This work conducted by Ola Kolawole in the laboratory of Christiane Wobus (University of Michigan)
CMX521: a Small Molecule Antiviral for Norovirus

- Nucleoside with pan-genotype activity
  - Targets a region of virus that is common to all strains
  - Acts via inhibition of viral polymerase activity

- Promising safety profile
  - Clean in studies of genotoxicity and mitotoxicity
  - IND-enabling nonclinical safety studies provide high safety margins for human exposure

- Preferential delivery to the cells that Norovirus infects?
  - Could improve odds of clinical efficacy: more drug in right place
  - Could improve odds of clinical safety: less drug in wrong place
Two Distinct Unmet Needs CMX521 May Address

- **Treatment** of Chronic Norovirus Infection
  - Transplant recipients and other symptomatic immunocompromised patients
  - Asymptomatic shedders
    - Food handlers, hospital/healthcare workers who may be source of outbreaks

- **Prevention** of Acute Norovirus Infection
  - Protect individuals from a potential outbreak (hospitals, long-term care facilities etc.)
  - Significantly reduce the economic impact of outbreaks
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