High and continuous exposure of Laninamivir \textit{in vivo}, generating from Laninamivir octanoate (CS-8958), a long-acting neuraminidase inhibitor for influenza may suppress to generate low-susceptible mutants.

Makoto Yamashita, Shuku Kubo and Masayo Kakuta.

Daiichi Sankyo Co. Ltd., Biological Res. Labs., Tokyo, Japan.
Laninamivir and Laninamivir Octanoate, a long-acting neuraminidase inhibitor

Launched on Oct. 2010 in Japan as a single inhalation drug to complete influenza treatment
Inhibitory activities of neuraminidase inhibitors to H274Y oseltamivir-resistant viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutation in NA</th>
<th>Laninamivir</th>
<th>Zanamivir</th>
<th>Oseltamivir carboxylate</th>
<th>Peramivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>seasonal H1N1</td>
<td>Wt</td>
<td>3.03</td>
<td>2.70</td>
<td>2.28</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>H274Y</td>
<td>5.62</td>
<td>3.05</td>
<td>755</td>
<td>59.2</td>
</tr>
<tr>
<td>(H1N1)pdm09</td>
<td>Wt</td>
<td>0.74</td>
<td>1.20</td>
<td>1.90</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>H274Y</td>
<td>1.14</td>
<td>1.04</td>
<td>1146</td>
<td>124</td>
</tr>
<tr>
<td>HPAIVI H5N1</td>
<td>Wt (2)</td>
<td>0.28-0.32</td>
<td>0.15-0.72</td>
<td>0.31-0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H274Y (2)</td>
<td>1.1-2.1</td>
<td>0.22-0.68</td>
<td>430-1100</td>
<td></td>
</tr>
</tbody>
</table>

**Laninamivir** is an active metabolite of Laninamivir Octanoate. **Oseltamivir carboxylate** is an active metabolite of Oseltamivir.

Cross-resistant to H274Y mutants

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Laninamivir octanoate: Inavir® inhalation, once
Zanamivir: Relenza® inhalation, twice daily x 5 days
Oseltamivir: Tamiflu® oral, twice daily x 5 days
Peramivir: Rapiacta® intravenous, once

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Yamashita et al. AAC (2009)
Nguyen et al AAC (2010)
CS-8958 (Laninamivir octanoate) dramatically increases survival rate of mouse infected with influenza virus.
Virus load reduction in mice infected with wt and resistant viruses by **CS-8958 (single, in)** and **oseltamivir (repeated, or)**

Kubo et al. AAC (2010)

**Virus titer (log_{10} pfu/lung)**

**Wild type virus**
- Control
- Oseltamivir: 1.0 mg/kg
- Oseltamivir: 10 mg/kg
- CS-8958: 0.027 mg/kg
- CS-8958: 0.080 mg/kg

**Oseltamivir-resistant virus (H274Y)**
- Control
- Oseltamivir: 10 mg/kg
- CS-8958: 0.027 mg/kg
- CS-8958: 0.080 mg/kg

**Mouse**: BALB/c, female, 6w
**Infection**: A/PR/8/34, 30pfu
**N = 3 (each time point)**
Laninamivir showed potent neuraminidase inhibitory activities against the following viruses:

- seasonal (H1N1, H3N2 and B)
- (H1N1)pdm09
- highly pathogenic avian influenza (H5N1)
- Oseltamivir-resistant seasonal H1N1 and (H1N1)pdm09
- all subtypes with N1-N9

Summary of non-clinical in vitro experiment of Laninamivir

Yamashita et al. AAC (2009)
Ito et al. Nature (2009)
Kiso et al. PLoS Pathog (2010a)
Kiso et al. PLoS Pathog (2010b)
Kubo et al. JJA (2010)
Summary of non-clinical in vivo experiment of CS-8958, Laninamivir octanoate

CS-8958 showed potent efficacy in mouse, ferret guinea pig infection model:

- seasonal (H1N1, H3N2 and B)
- (H1N1)pdm09
- highly pathogenic avian influenza (H5N1)
- Oseltamivir-resistant seasonal H1N1 and pdm(H1N1)2009

Kubo et al. AAC (2010)
Ito et al. Nature (2009)
Kiso et al. PLoS Pathog (2010a)
Kiso et al. PLoS Pathog (2010b)
Kubo et al. JJA (2010)
Why is CS-8958 effective by a single intranasal administration?

Two unique characteristics of CS-8958 giving a long action

1. Pharmacokinetic feature
   CS-8958 administered intranasally retains in mice for a long time as an active metabolite, laninamivir.

2. Strong NA-binding feature
   Laninamivir strongly binds to neuraminidase of influenza viruses.
High and continuous exposure of laninamivir after laninamivir octanoate (CS-8958) administration

CS-8958: 0.24 mg/kg, *in vivo*
Less than clinical doses

Laninamivir exists in lungs 70-5100 times higher than IC50s even 120 hours after in administration of CS-8958.

<table>
<thead>
<tr>
<th>Type/subtype</th>
<th>IC50 (range)</th>
<th>Ratio of Conc/IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1N1</td>
<td>2.09 (1.29-5.07)</td>
<td>3700-530</td>
</tr>
<tr>
<td>(H1N1)pdm09</td>
<td>1.50 (1.21-2.11)</td>
<td>5100-730</td>
</tr>
<tr>
<td>H3N2</td>
<td>14.2 (7.09-38.8)</td>
<td>540-77</td>
</tr>
<tr>
<td>B</td>
<td>15.9 (10.4-31.4)</td>
<td>480-70</td>
</tr>
</tbody>
</table>

Yamashita et al. AAC (2009)
Kubo et al. JJA (2010)
Koyama et al. AAC (2009)
Question

Does high and continuous exposure of laninamivir generated in lungs after administration of CS-8958 suppress to generate low-susceptible mutants?
Isolation of *in vivo* resistant mutants in mice infection model

1. Infected with A/PR/8/34 (H1N1).
2. Treated with CS-8958 once (*in*) or oseltamivir *bid* for 5 days (*oral*) which gave a similar virus reduction profile in the lungs.
3. Plaque formation from the lungs.
4. Virus isolation from the plaques of the lungs excised at 7 days *poi* which was the last day to detect viruses.
6. Sequence analysis of NA gene of the isolates with IC$_{50}$ reduced by more than 2 times.
Mice were infected with A/PR/8/34 (30pfu) at day 0.

Isolation of viruses at 7 days postinfection

Virus load in lungs (log_{10}pfu/lungs)

- saline
- CS-8958 (0.08 mg/kg, single, intranasal)
- Oseltamivir (1 mg/kg, twice daily for 5 days, oral)

Kubo et al. AAC (2012)
Estimated PK profiles of the two compounds under this experimental conditions

Laninamivir:
- From Koyama et al AAC (2009)

Oseltamivir carboxylate:
- From NDA data

IC$_{50}$ range

CS-8958 0.08mg/kg

Oseltamivir 1mg/kg

Hours: 0 24 48 72 96 120 168 (7 days)
## Generation of viruses resistant to NA inhibitors \textit{in vivo}

<table>
<thead>
<tr>
<th>treated with</th>
<th>CS-8958</th>
<th>Oseltamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse No. 1</td>
<td>1163</td>
<td>1127</td>
</tr>
<tr>
<td></td>
<td>(2358)</td>
<td>(2308)</td>
</tr>
<tr>
<td>mouse No. 2</td>
<td>1195</td>
<td>1181</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. viruses picked up</td>
<td>1163</td>
<td>1127</td>
</tr>
<tr>
<td></td>
<td>(2358)</td>
<td>(2308)</td>
</tr>
<tr>
<td>No. viruses with IC$_{50}$ reduced by more than 2 times</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Mutation in NA</td>
<td>-</td>
<td>V116A</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>R152K</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R371K</td>
</tr>
</tbody>
</table>

Kubo et al. AAC (2012)
NA inhibitory activities of NA inhibitors to oseltamivir-low susceptible viruses generated *in vivo*

<table>
<thead>
<tr>
<th>Mutation in NA</th>
<th>Oseltamivir carboxylate</th>
<th></th>
<th>Laninamivir</th>
<th></th>
<th>Zanamivir</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (nM)</td>
<td>-fold</td>
<td>IC$_{50}$ (nM)</td>
<td>-fold</td>
<td>IC$_{50}$ (nM)</td>
<td>-fold</td>
</tr>
<tr>
<td>A/PR/8/34</td>
<td>Parent virus (wt)</td>
<td>1.82</td>
<td>0.779</td>
<td>0.703</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V116A</td>
<td>4.24</td>
<td>0.352</td>
<td>1.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G137S</td>
<td>3.68</td>
<td>0.389</td>
<td>1.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low susceptible virus</td>
<td>R152K</td>
<td>31.3</td>
<td>3.54</td>
<td>4.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R371K</td>
<td>26.1</td>
<td>0.751</td>
<td>3.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kubo et al. AAC (2012)
Summary of resistant virus generation

\textit{In vitro} (data not shown in today’s presentation)
Laninamivir is a compound which generate low-susceptible mutants less frequently compared with oseltamivir carboxylate.
It’s probably due to a structural similarity of Laninamivir to sialic acid, natural substrate of neuraminidase.

\textit{In vivo}
From mice administered CS-8958 intranasally, no low-susceptible viruses were obtained.
It’s possible due to high and long exposure of Laninamivir in lungs which is a target organ of influenza virus infection.

CS-8958 will suppress to generate its resistant viruses in \textit{in vivo} because of the intrinsic character and the unique pharmacokinetic features.
Favorable characters of CS-8958, Laninamivir octanoate for treatment of influenza

**CS-8958** (20mg or 40mg, inhalation, once) to complete a treatment
- high patient compliance
- low frequency to generate resistant mutants

Licensed NA inhibitors

Zanamivir (10 mg×10, inhalation)
Oseltamivir (75 mg×10, oral)
Peramivir (300 mg, intravenous)

\{ twice daily for 5 days
\}
once
Thank you for your kind attention.