Mechanism of resistance to neuraminidase inhibitors of influenza A viruses

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Schematic representation of influenza

- Neuraminidase
- Haemagglutinin
- Segmented negative single strand RNA
- Protein M₂ (Type A only)
Putative antiviral targets

- Neuraminidase inhibitors
- Haemagglutinin inhibitors
- Ion channel (M2) blockers
- Nucleoprotein inhibitors
- Polymerase inhibitors
Influenza Neuraminidase Glycoprotein

- Cytoplasmic tail
- Trans-membrane domain
- stalk
- Head
Commercially available influenza virus neuraminidase inhibitors

Zanamivir (Relenza®)
Oseltamivir (Tamiflu®)
Peramivir (not shown)
NAI Resistance: Preliminary data
Mutations associated with resistance and susceptibility to NAIs

In vitro data from LV Gubavera, Vir Res 2004:

<table>
<thead>
<tr>
<th>function</th>
<th>position</th>
<th>virus</th>
<th>treatment</th>
<th>oseltamivir</th>
<th>zanamivir</th>
<th>peramivir</th>
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</thead>
<tbody>
<tr>
<td>Framework</td>
<td>N119V</td>
<td>H3N2</td>
<td>Oseltamivir</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td></td>
<td>H274Y</td>
<td>H1N1</td>
<td>Oseltamivir</td>
<td>R</td>
<td>S</td>
<td>R</td>
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<td></td>
<td></td>
<td>H5N1</td>
<td>Oseltamivir</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>D198N</td>
<td>B</td>
<td>Oseltamivir</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
<td>Catalytic</td>
<td>R292K</td>
<td>H3N2</td>
<td>Oseltamivir</td>
<td>R</td>
<td>Nd/R</td>
<td>nd</td>
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<tr>
<td></td>
<td>R152K</td>
<td>B</td>
<td>Zanamivir</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>
Resistance to neuraminidase inhibitors in influenza A

- R152K – Catalytic mutation
- E119V – Framework mutation
- D198N – Framework mutation
- H274Y – Framework mutation
- R292K – Catalytic mutation
- Asn294Ser – Framework mutation?
Mechanisms of Influenza virus resistance to neuraminidase inhibitors

- Binding tight
  - Release enhance (N2)
  - NA dependence decrease
  - Resistant to inhibitor
  - Fitness ++++

- Binding Tight or moderate
  - Release enhance (N2)
  - NA dependence decrease
  - Decrease inhibitor efficiency
  - Fitness +/-

- Binding moderate
  - Release enhance (N2)
  - Fitness ++
A H5N1 and susceptibility to NAIs
Review about level of resistance

1- NISM (Antivir Res, 2005) : 0,4%
3- Ferraris et al (Antivir Res, 2005) : 0,9%
4- deJong et al (NEJM, 2005) : 2 cases of A H5N1
5- Whitley et al (Pediatr Infect Dis, 2001) : 4% in children
6- Evolution of the susceptibility of A H5N1 strains
7- Ison et al (JID 2006) : analysis of 3 cases with resistant strains
8- Others...
Influenza A H5N1 as in July 2006

- Clade 1
- Clade 2
Phylogenetic analysis of A H5N1 Ha1

From A Hay, EISS meeting 2006, Malta
Level of susceptibility of clinical isolates

• In vitro, H5N1 isolates from poultry and humans are susceptible to both NAIs
• Viruses from the 2 clades may have different susceptibility patterns:
  – Clade 1 seems to be less susceptible to NAIs than Clade 2 viruses
  – Clade 1 is resistant to Amantadine while some Clade 2 viruses are susceptible
Level of transmissibility of resistant isolates

- Amantadine resistant isolates are highly transmissible
- 119 mutants have been shown to be transmissible in ferrets (Matrosovich et al, 2006)
- 274 mutants seem to have been transmitted from a H5N1 infected child (deJong et al, 2005)
- In highly replicating viruses such as A H5N1, fitness reduction due to NAI resistance may not impair transmissibility
NAI Resistance:
recent data from the literature
Review about level of resistance

1- NISM (Antivir Res, 2005) : 0,4%
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No concensus on what is the cut-off (threshold) for resistance
1- Reduction of virus Fitness

2- Reduction of virus transmission/infectivity (but...)

3- No transfer of resistance (N1 to N2; H1N1 to H5N1)

4- No cross-resistance with M2 blockers

5- Different level of resistance according to the substitutions (Oselta vs Zana; in vitro data)

6- Quasi-species and selection of resistance

7- Impact of prophylactic treatments ?

8- Different level of resistance depending on Na sub-type ?
Surveillance of emerging NAI-resistant H1N1 & H3N2 viruses
Fluorometric Neuraminidase Assay

- Neuraminidase activity assay
- Neuraminidase activity inhibition assay

MUN:
2’-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid

1 H – 37°C

Ex: 355 nm
Em: 460 nm

Zanamivir or Oseltamivir

15 min – 37°C
Neuraminidase inhibition fluorescence test on wild type virus and R292K by **Zanamivir**

Fluorometric inhibition assay by Zanamivir

| Neuraminidase inhibition fluorescence test on wild type virus and R292K by **Oseltamivir** |

| Fluorometric inhibition assay by Oseltamivir |

<table>
<thead>
<tr>
<th>IC50 nM</th>
<th>Zanamivir</th>
<th>Oseltamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A/Sydney/05/97</strong></td>
<td>N 28 Mean 3.7 SD 1.8 CV 48.7</td>
<td>N 28 Mean 0.67 SD 0.37 CV 55.3</td>
</tr>
<tr>
<td><strong>R292K</strong></td>
<td>N 28 Mean 22.86 SD 7.66 CV 33.5</td>
<td>N 28 Mean 149.14 SD 8728 CV 58.5</td>
</tr>
</tbody>
</table>
Neuraminidase inhibition fluorescence test on B/Beijing/1/87 Wild type and R152K mutant virus by Zanamivir

Fluorometric inhibition assay by Zanamivir

Fluorometric inhibition assay by Oseltamivir

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<tr>
<th>IC50 nM</th>
<th>Zanamivir</th>
<th>Oseltamivir</th>
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<tbody>
<tr>
<td>B/Beijing/1/87 WT</td>
<td>6.7</td>
<td>15.6</td>
</tr>
<tr>
<td>Mean</td>
<td>2.32</td>
<td>5.15</td>
</tr>
<tr>
<td>SD</td>
<td>34.95</td>
<td>83.1</td>
</tr>
<tr>
<td>CV</td>
<td>19</td>
<td>33.1</td>
</tr>
</tbody>
</table>

| B/Beijing/1/87 R | 6274      | 438.2       |
| Mean            | 5363      | 83.1        |
| SD              | 85.5      | 19          |
| CV              | 34.95     | 33.1        |
Sensitivity of influenza viruses to zanamivir and oseltamivir

A study performed on 1180 viruses circulating in France (2002-2006) prior to and after the introduction of NAIs in clinical practice with a high throughput assay

**IC50 Zanamivir**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Mean (nM)</th>
<th>Max (nM)</th>
<th>Upper limit (nM)</th>
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</thead>
<tbody>
<tr>
<td>A/H3N2</td>
<td>2.28</td>
<td>5.97</td>
<td>5.6</td>
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<tr>
<td>A/H1N2</td>
<td>3.09</td>
<td>6.70</td>
<td>6.4</td>
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<tr>
<td>A/H1N1</td>
<td>0.92</td>
<td>1.09</td>
<td>1.2</td>
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<tr>
<td>B</td>
<td>4.19</td>
<td>11.90</td>
<td>13.4</td>
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**IC50 Oseltamivir**

<table>
<thead>
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<th>Subtype</th>
<th>Mean (nM)</th>
<th>Max (nM)</th>
<th>Upper limit (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/H3N2</td>
<td>0.67</td>
<td>2.38</td>
<td>2.2</td>
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<tr>
<td>A/H1N2</td>
<td>0.9</td>
<td>1.62</td>
<td>1.7</td>
</tr>
<tr>
<td>A/H1N1</td>
<td>1.34</td>
<td>3.02</td>
<td>33.5</td>
</tr>
<tr>
<td>B</td>
<td>12.99</td>
<td>30.20</td>
<td>33.5</td>
</tr>
</tbody>
</table>

**Susceptibility values**

- Zanamivir < Oseltamivir
- Oseltamivir < Zanamivir
Detection of influenza virus with decreased susceptibility to neuraminidase inhibitors

1- Outliers viruses :

2- Resistant viruses :
H3 strains were tested with a fluorometric neuraminidase inhibition assay, underlining differences in IC₅₀ range according to the anti-neuraminidase drug.

- 3 viruses were isolated with upper limit oseltamivir IC₅₀ values
- 8 viruses were isolated with upper limit zanamivir IC₅₀ values
- 7 point mutations were isolated from the neuraminidase sequence of these outliers viruses: A18S; L23F; C42F; R143V; E199K; S332F; K421N

NA substitutions to be analysed by Reverse Genetics.
Neuraminidase mutations: association with resistance

- **E119V**
- **H274Y**
- **R292K**
- **R152K**
- **D198N**
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