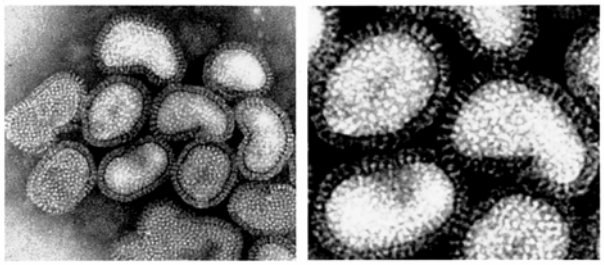
INFLUENZA VIRUS

Adapté en partie des exposés de la Chaire Franqui 2003
"Antiviral drugs and Discoveries in Medicine"
Prof. E. De Clercq, KU-Leuven
http://www.md.ucl.ac.be/chaire-francqui/

et de l'exposé du Dr R. Snacken
Institut Scientifique de Santé Publique, Bruxelles
au Séminaire de Pathologie Infectieuse de l'UCL
27-10-2006 – http://www.md.ucl.ac.be/seminfect/resume

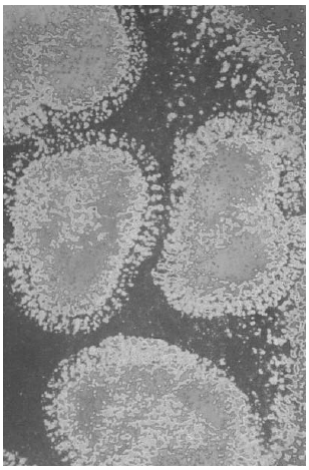
Influenza virus



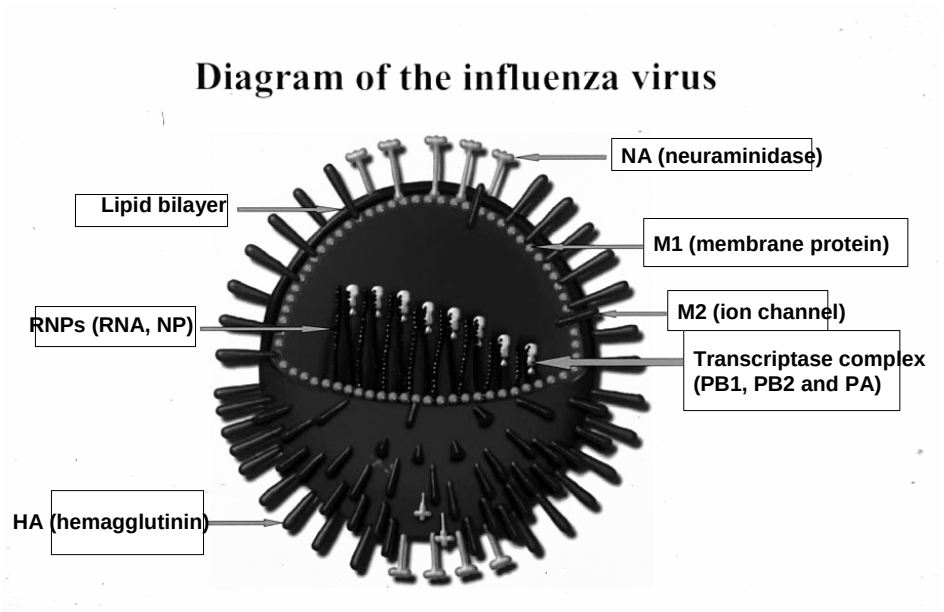
Electron micrographs of purified influenza virions. Hemagglutinin (HA) and neuraminidase (NA) can be seen on the envelope of viral particles. Ribonucleoproteins (RNPs) are located inside the virions.

http://www.virology.net/Big_Virology/BVRNAortho.html

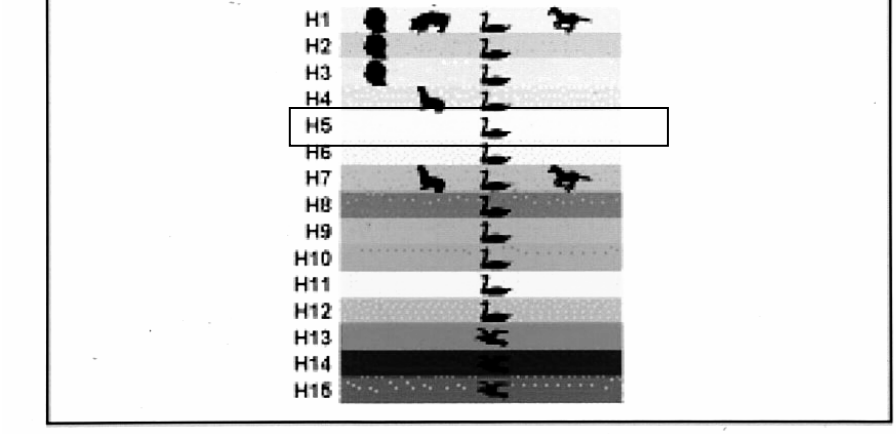
Influenza



Layne *et al*., Science 293: 1729 (2001)

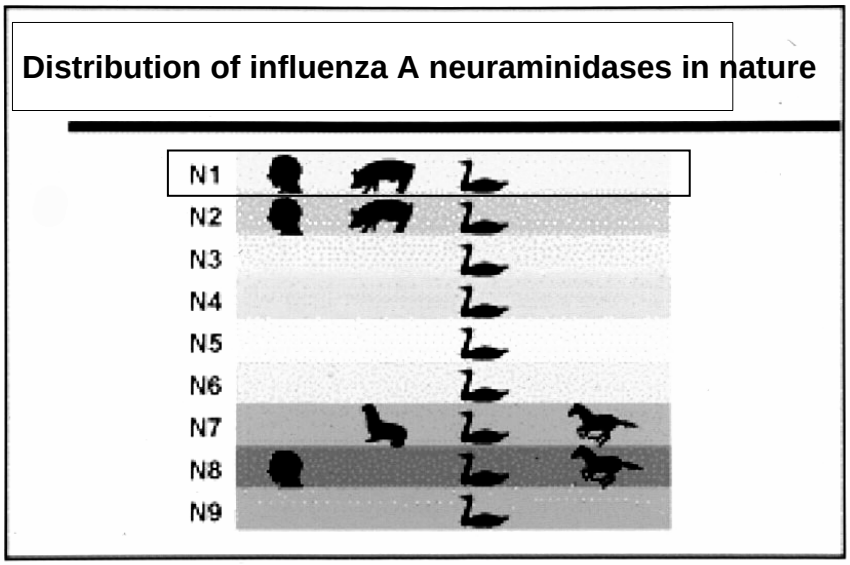


Distribution of influenza A hemagglutinins in nature



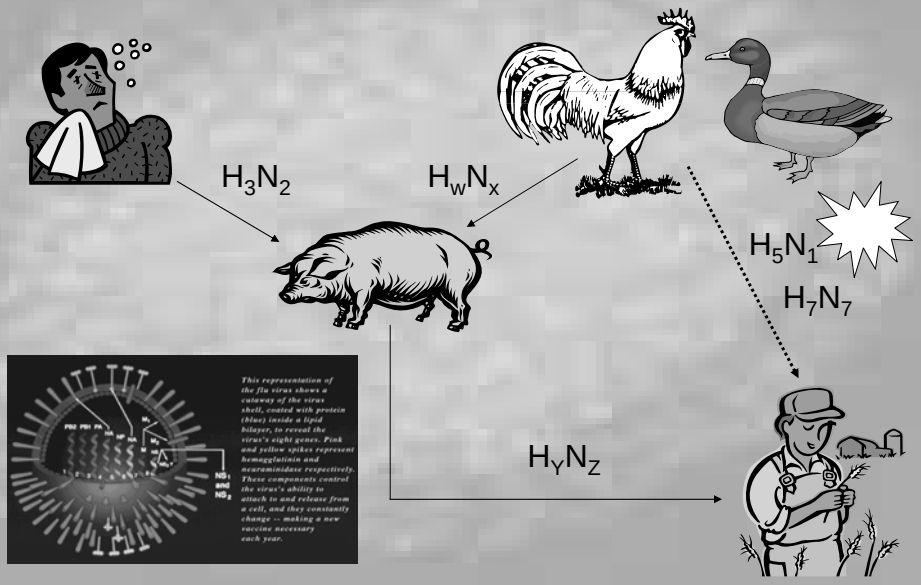
http://www.brown.edu/Courses/Bio_160/Projects1999/flu/mechanism.html

Distribution of influenza A neuraminidases in nature



http://www.brown.edu/Courses/Bio_160/Projects1999/flu/mechanism.html

Influenza A Virus Shift



Influenza A Pandemics

1889-90	A/H2N8	
1900-03	A/H3N8	
1918-19	A/H1N1	*Spanish Flu*
1957-58	A/H2N2	*Asian Flu*
1968-69	A/H3N2	*Hong Kong Flu*
(1977-78)	A/H1N1	*Russian Flu*

Australian Red Cross 1918

Best candidates for the next pandemic :

H2 or a HA (H5 or H7) with a human receptor binding protein

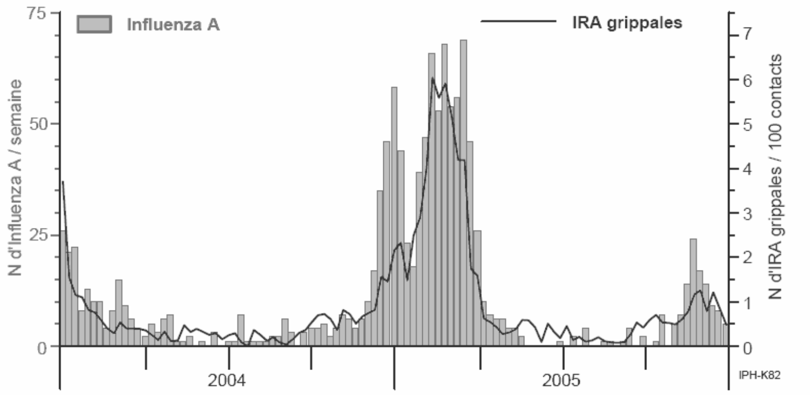
H9N2?

Evolution des "maladies grippales" et de l'isolement d'*Influenza A*

Laboratoires Vigies — **Influenza A**

- l'évolution du nombre d'Influenza A est comparable à celle du nombre d'IRA grippales enregistrées par le réseau de médecins généralistes participant au programme de surveillance des IRA et de la grippe (figure 7).

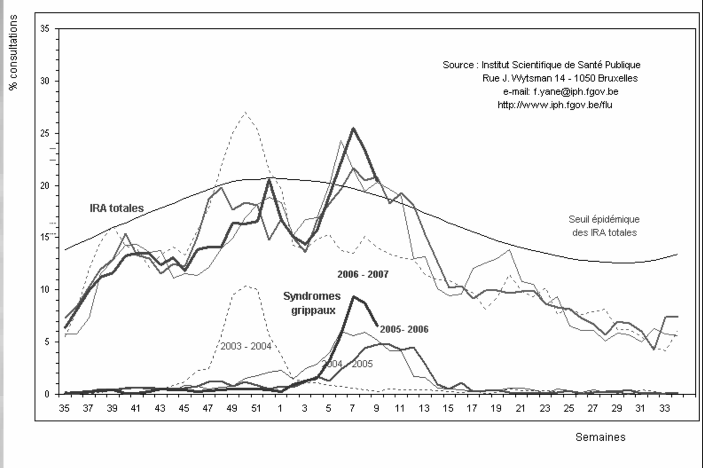
Figure 7 : Influenza A : comparaison entre l'évolution par semaine du nombre d'Influenza A et celle des I.R.A. grippales (2004-2005)



http://www.iph.fgov.be/epidemio/epifr/plabfr/plabanfr/05_082f_v.pdf

Epidémiologie annuelle des infections par *Influenza*

Evolution du pourcentage de grippes et d'infections respiratoires aiguës (IRA) totales enregistrées par les médecins sentinelles chez leurs patients



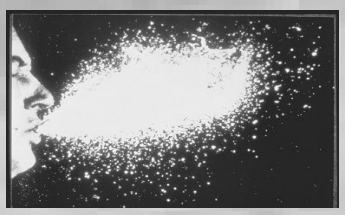
Semaine N°	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4
Nombre de cas de grippe confirmés par le Centre national de la Grippe (prélèvements effectués par les médecins sentinelles)																	
Influenza A	0	0	0	0	0	0	0	0	0	0	0	0	1	2	8	34	44
A non sous-typé																1	2
A/H3													1	2	7	33	42
A/H1															1		
Influenza B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nombre de cas de grippe confirmés par les hôpitaux universitaires																	
Influenza A	0	0	0	0	0	1	0	0	1	1	2	1	9	17	16	31	67
A non sous-typé											1	1	4	17	16	31	67
A/H3									1	1	1		5				
A/H1						1											
Influenza B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Semaine N°	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Nombre de cas de grippe confirmés par le Centre national de la Grippe (prélèvements effectués par les médecins sentinelles)																	
Influenza A	102	149	115	102	41												
A non sous-typé	4	15	5	38	41												
A/H3	96	133	108	64													
A/H1	2	1	2														
Influenza B	0	0	0	0	1												
Nombre de cas de grippe confirmés par les hôpitaux universitaires																	
Influenza A	155	199	206	94	15												
A non sous-typé	155	199	206	94	15												
A/H3																	
A/H1																	
Influenza B	0	4	0	0	0												

http://www.iph.fgov.be/flu/FR/22FR.htm

Transmission Routes of Influenza

1. Droplets

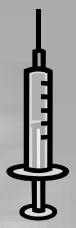


2. Airborne droplet nuclei

3. Fomites

A fomite is any inanimate object or substance capable of absorbing infectious organisms (such as germs or parasites) and hence transferring them from one individual to another.

Recommendations for Preventing Influenza



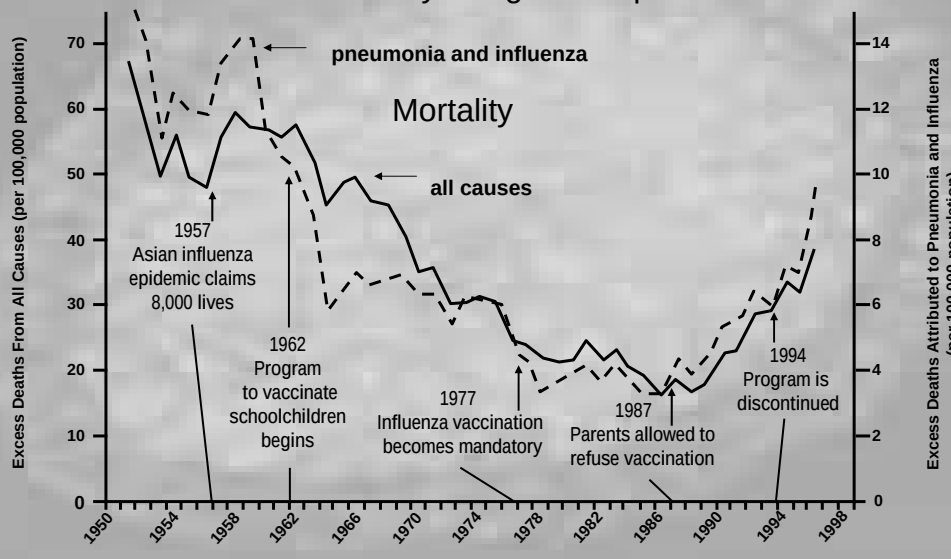
Current recommendations are essentially targeted to :

- Persons at high risk of complications (>65 y, institutionalized persons, underlying chronic condition from the age of 6 month : heart, lung, liver, kydney, diabetes and other immunologically frail people)
- Persons who can transmit the disease to high risk persons

(medical staff, households, …)

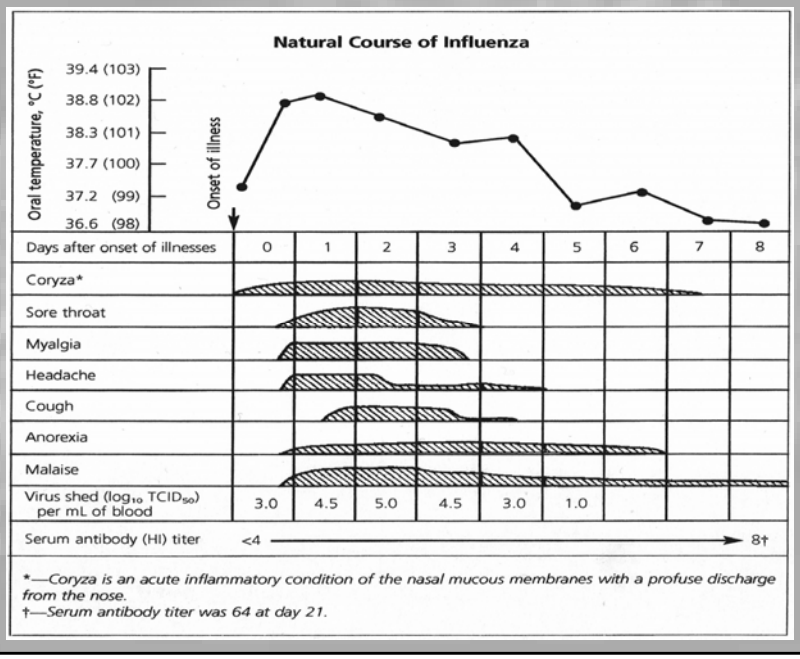
- Anywhone who whishes to be vaccinated
- These recommendations were recently extended in Sept 2005

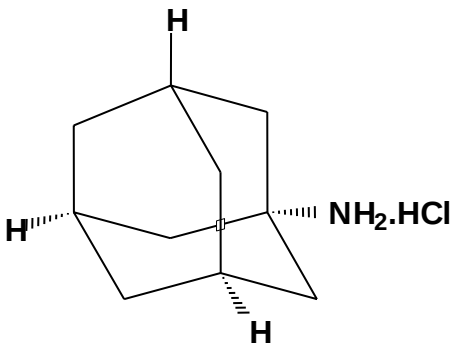
Herd Immunity : Flu Vaccination in children and mortality all ages in Japan



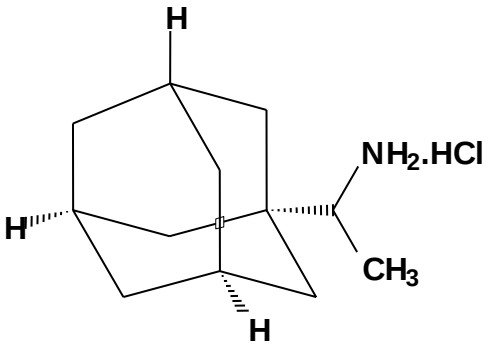
Reichert TA et al. *N Engl J Med*. 2001;344:889-896

Clinical Course of Influenza



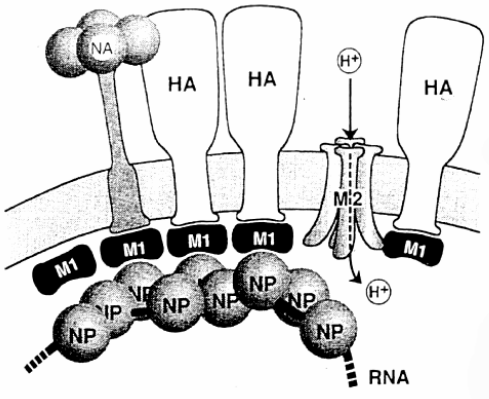


Amantadine

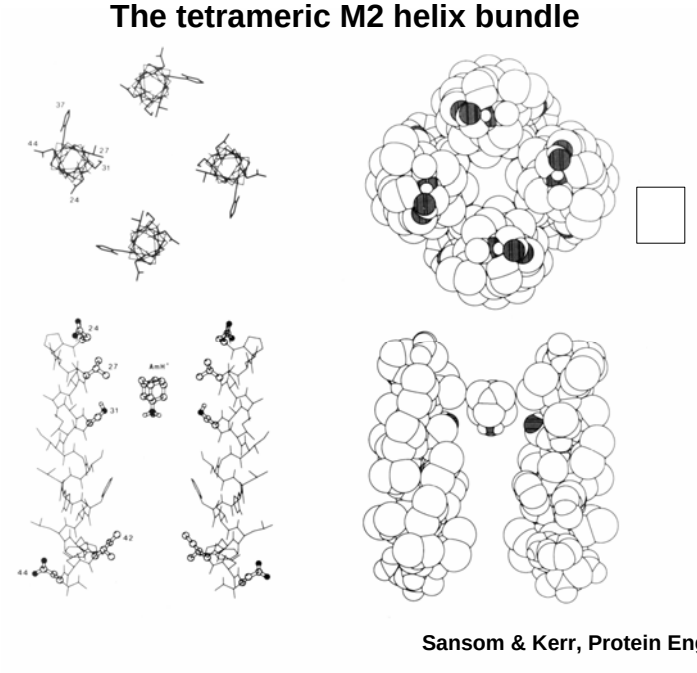


Rimantadine

Amantadine/rimantadine:
mechanism of action limited to influenza A viruses



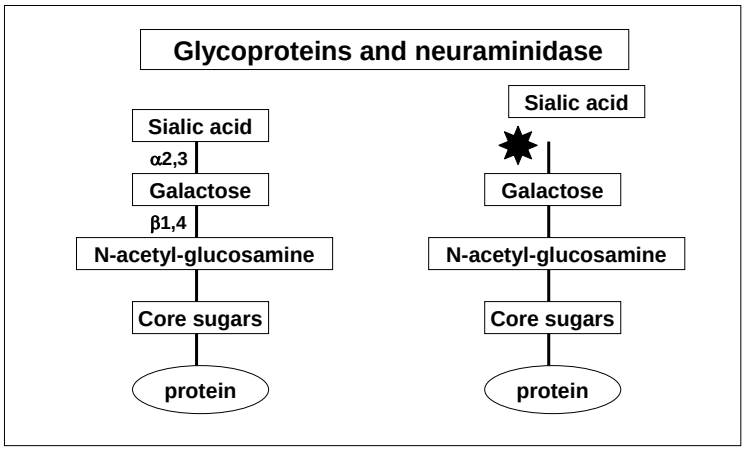
The tetrameric M2 helix bundle



Sansom & Kerr, Protein Eng. 6: 65-74 (1993)

Neuraminidase (NA):

Cleaves sialic acid from cell-surface glycoprotein

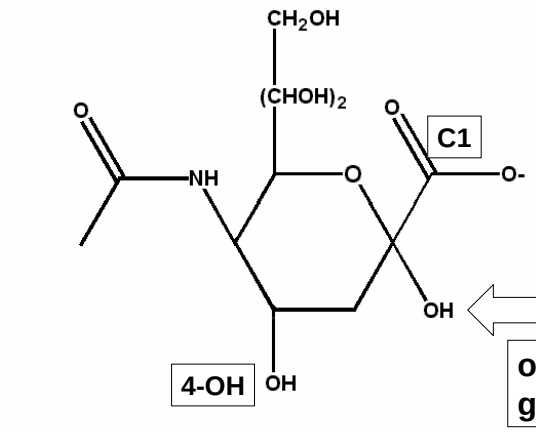


Influenza virus neuraminidase

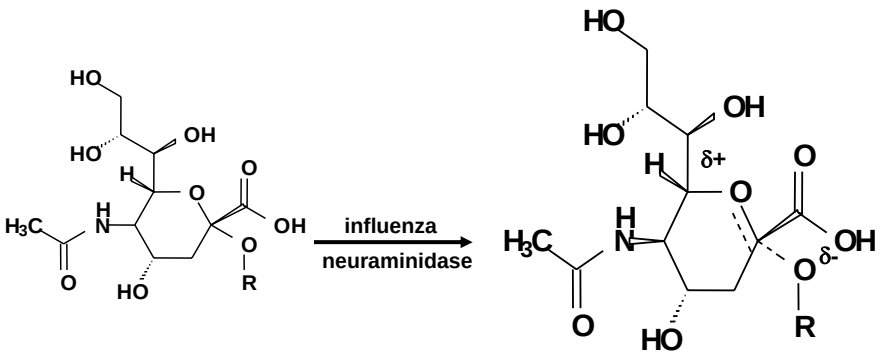
Functions:

- **removes terminal sialic acid residues**
- **promotes release of virus particles from the cells**
- **destroys cellular receptors recognized by hemagglutinin**
- **prevents virus aggregation at the cell surface**
- **prevents viral inactivation by respiratory mucus**

Sialic acid...



osidic linkage with galactose (2 → 3)

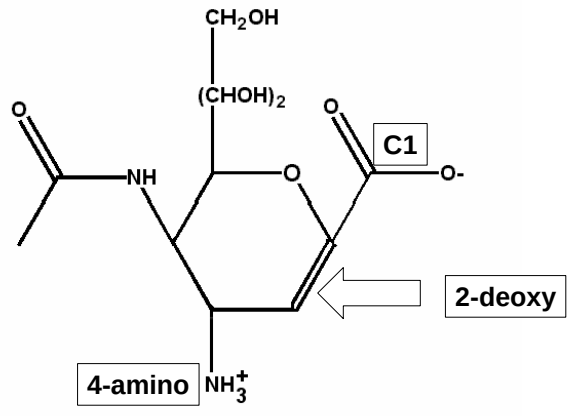


Sialyl α-glycoside
R = glycoprotein

Transition state

Abdel-Magid *et al*., Curr. Opin. Drug Discov. Dev. 4: 776-791 (2001)

First inhibitor of neuraminidase… (1969)



2,4-dideoxy-2,3-didehydro-4-amino-D-*N-acetylneuraminic acid*

Meindl et al., Hoppe-Seyler's Z. Physiol. Chem., 350:1088-1092, 1969

From 1969 to 1993...

- **2,3-dideoxy-2,3-didehydro-4-amino-D-*N*-acetylneuraminic acid**
  K_i ~ 0.01 mM vs K_m for sialic acid ~ 1mM

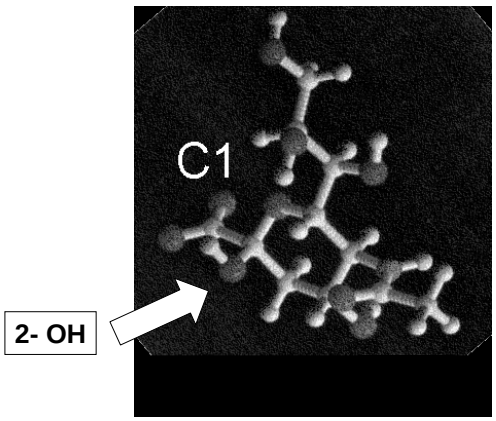
  ⇒ **does not work...**

- **1983: structure of neuraminidase at 2.9 Å resolution**
  several residues at catalytic site are constant
  antigenic sites are highly variable…

  (Colman et al., Nature 303:41-44, 1983)

  ⇒ **Can you visualize the catalytic site ?**

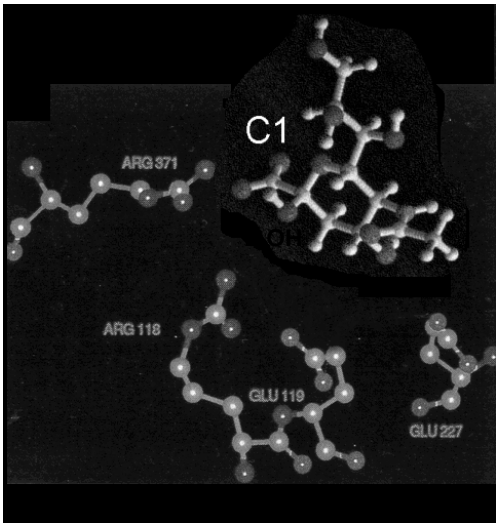
From sialic acid to zanamivir… (1)



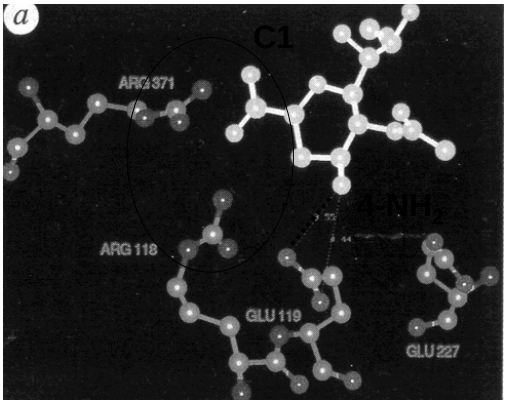
acide sialique ou N-acétyl-neuraminique

Dfrom sialic acid to zanamivir… (2)

sialic acid binds through its C1 carboxylate to Arg 371



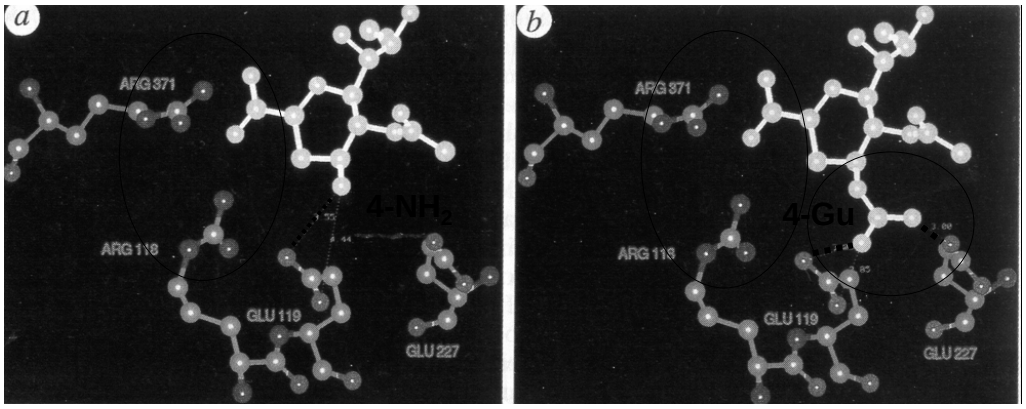
From sialic acid to zanamivir… (3)



4-deoxy-4-amino ...

résidues 119 et 227 are highly conserved...

From sialic acid to zanamivir… (4)



4-deoxy-4-amino ...

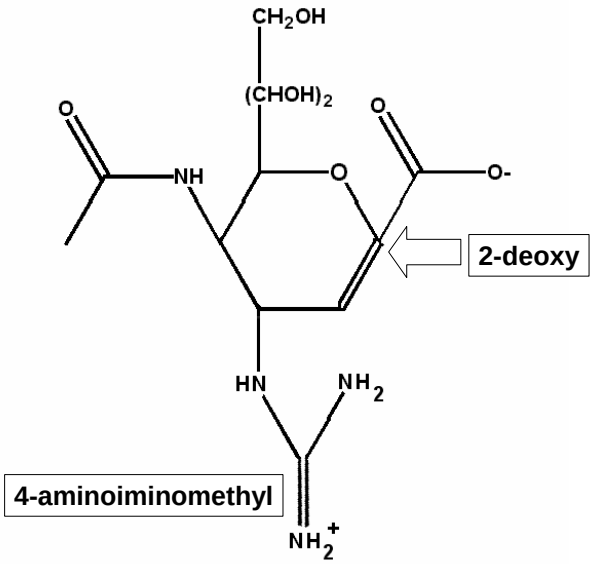
4-deoxy-4guanidino...

both residues 119 and 227 are now involved and 199 is much closer….

Zanamivir...
(1993)

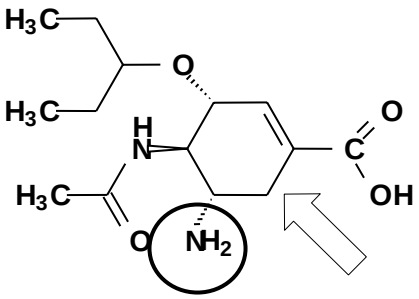
2,4-dideoxy-2,3-didehydro-4-guanidino-D-*N-acetylneuraminic acid*

von Itzstein et al.,
Nature 363: 418-423, 1993

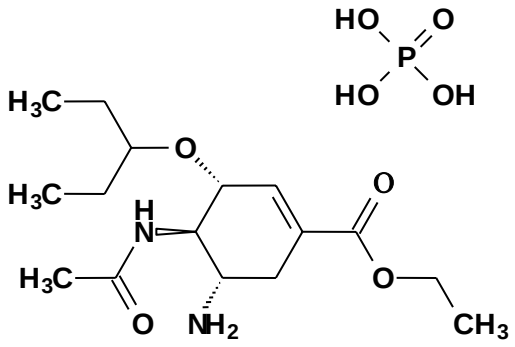


Zanamivir

- active against both influenza A and B
- IC_{50} : 0.21-2.6 ng/ml for influenza neuraminidase
- efficacy demonstrated in mouse and ferret models for influenza (upon topical administration)
- has to be administered by inhalation : 10 mg bid
- therapeutically effective (5 days) : significant reduction in duration of illness
- prophylactically effective (4 weeks) : significant reduction in number of ill subjects
- well tolerated : clinical adverse events not different from placebo
- no evidence for emergence of drug-resistant virus

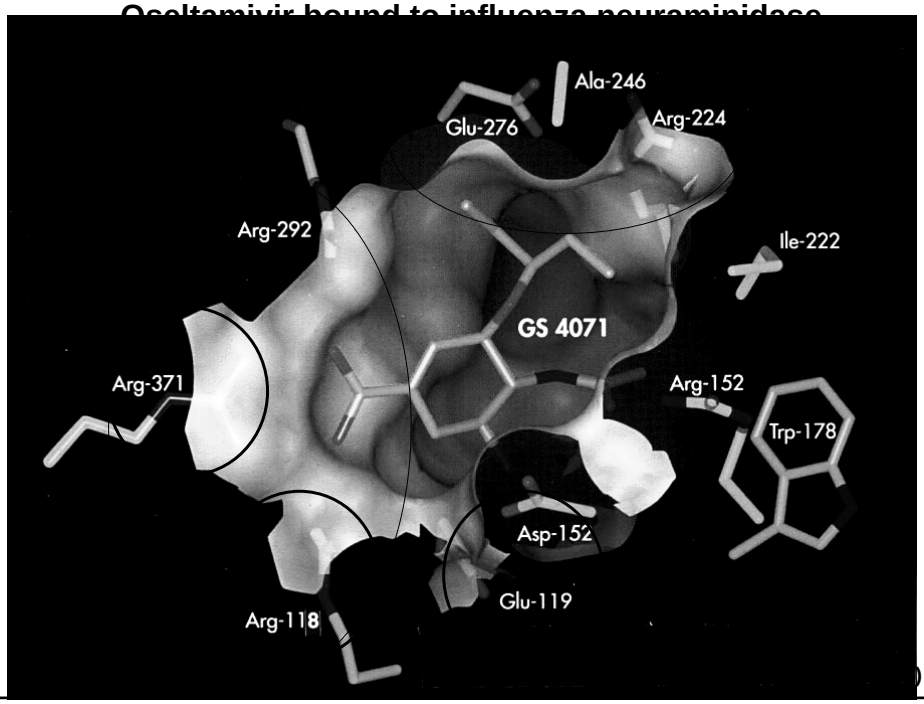


GS4071



Oseltamivir phosphate

Oseltamivir bound to influenza neuraminidase



Oseltamivir

- active against both influenza A and B
- IC_{50} : < 1 ng/ml for influenza neuraminidase
- efficacy demonstrated in mouse and ferret models for influenza (upon oral administration)
- can be administered orally : 75 or 150 mg bid
- therapeutically effective (5 days) in common human influenza : significant reduction in duration of illness
- **NOT** proven and probably poorly effective in humans contaminated with the H5N1 strain …. (resistance…)
- prophylactically effective (6 weeks) : significant reduction in number of ill subjects
- well tolerated : clinical adverse events not different from placebo

Stockpiling of Antivirals

Objective : 30% Belgian population should have access to treatment in 2008

RESISTANCE MUTATIONS TO NEURAMINIDASE INHIBITORS

Neuraminidase

119 Glu → Gly:

- **specific for zanamivir;**
- **Glu 119 interacts with guanidinium group of zanamivir**

292 Arg → Lys (R292K)**:**

- **found for zanamivir and reduces its activity**
- **causes resistance to oseltamivir**
  - ➔ **Arg 292 interacts with carboxylic acid group of zanamivir and oseltamivir but something more is needed with oseltamivir …**

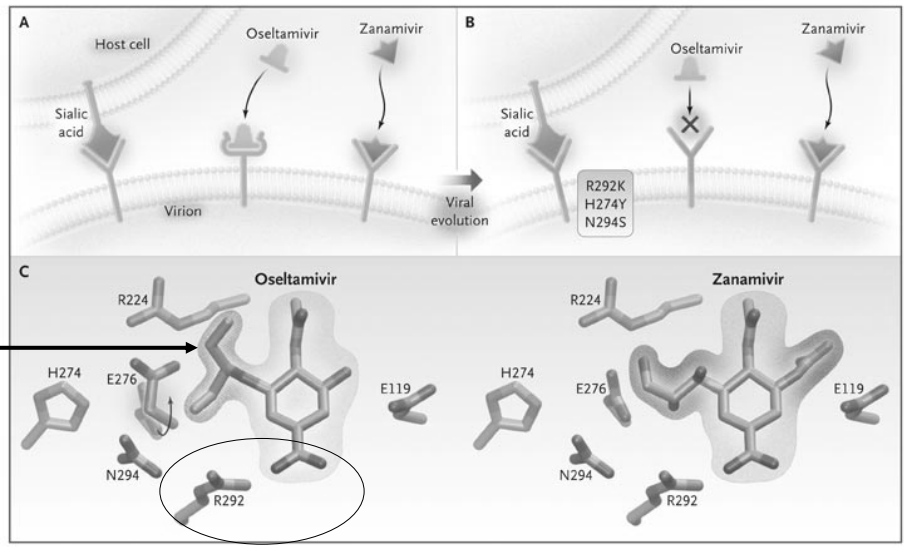
274 His → Tyr (H274Y) **and 294 Asn → Ser** (N294S)

- **cause resistance to oseltamivir but not zanamivir**

Hemagglutinin

Some mutations (i.e. 198 Thr → Ile) diminish affinity of hemagglutinin for its receptor

RESISTANCE MUTATIONS TO OSELTAMIVIR LEAVING ZANAMIVIR ACTIVE …



Moscona, A. N Engl J Med 2005;353:2633-2636

Mechanism of Resistance to Oseltamivir.

The neuraminidase active site changes shape to create a pocket for oseltamivir, whereas it accommodates zanamivir without such a change (Panel A). Any of several mutations may prevent the binding of oseltamivir by preventing the formation of this pocket (Panel B); the oseltamivir-resistant virus can nonetheless bind to the host-cell sialic acid receptor and to zanamivir. The pocket for oseltamivir, illustrated by key amino acids in Panel C, is created by the rotation of E276 and bonding of the amino acid to R224 — events that are prevented by the mutations R292K, N294S, and H274Y and therefore result in resistance to oseltamivir. An E119V mutation may permit the binding of a water molecule in the space created by the smaller valine, also interfering with oseltamivir binding. None of these mutations prevent the binding of zanamivir or of the natural sialic acid substrate.

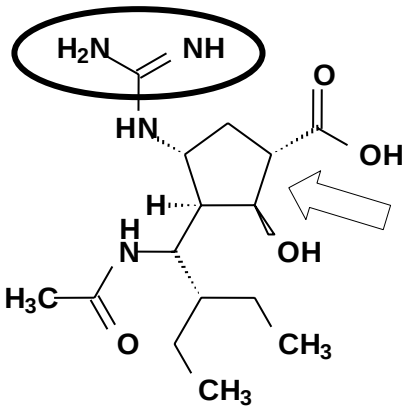
Possible benefits offered by neuraminidase inhibitors

Therapeutically:

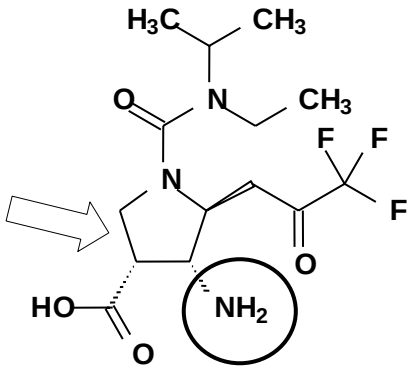
- **Reduction in illness duration by 1-2 days**
- **Reduction in risk-virus transmission to household or healthcare contacts**
- **Reduction in complications (sinusitis, bronchitis)**
- **Reduction in use of antibiotics**

Prophylactically:

- **Seasonal prevention of infection**



RWJ-270201

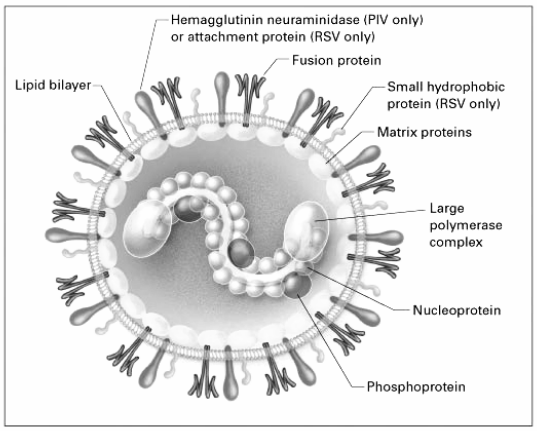


Wang *et al*., J. Med. Chem. 44: 1192-1201 (2001)

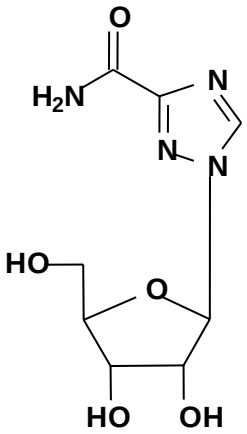
Smee *et al*., Antimicrob. Agents Chemother. 45: 743-748 (2001)
Sidwell *et al*., Antimicrob. Agents Chemother. 45: 749-757 (2001)

RESPIRATORY SYNCYTIAL VIRUS (RSV)

Respiratory Syncytial Virus (RSV) and Parainfluenza Virus (PIV)



Hall, N. Engl. J. Med. 344: 1917-1928 (2001)



Ribavirin

Virazole®

APPROVED ANTIVIRAL DRUGS FOR THE TREATMENT OF THE MAJOR RESPIRATORY TRACT VIRUS INFECTIONS in 2003

Adenoviruses : none

Picornaviruses

Entero : none

Rhino : none

Orthomyxoviruses

Influenza : Neuraminidase inhibitors: zanamivir, oseltamivir

: Amantadine and rimantadine (for influenza A only)

Paramyxoviruses

Parainfluenza : none

Respiratory syncytial virus : Ribavirin

SARS virus : none