Mechanism of resistance to neuraminidase inhibitors of influenza A viruses

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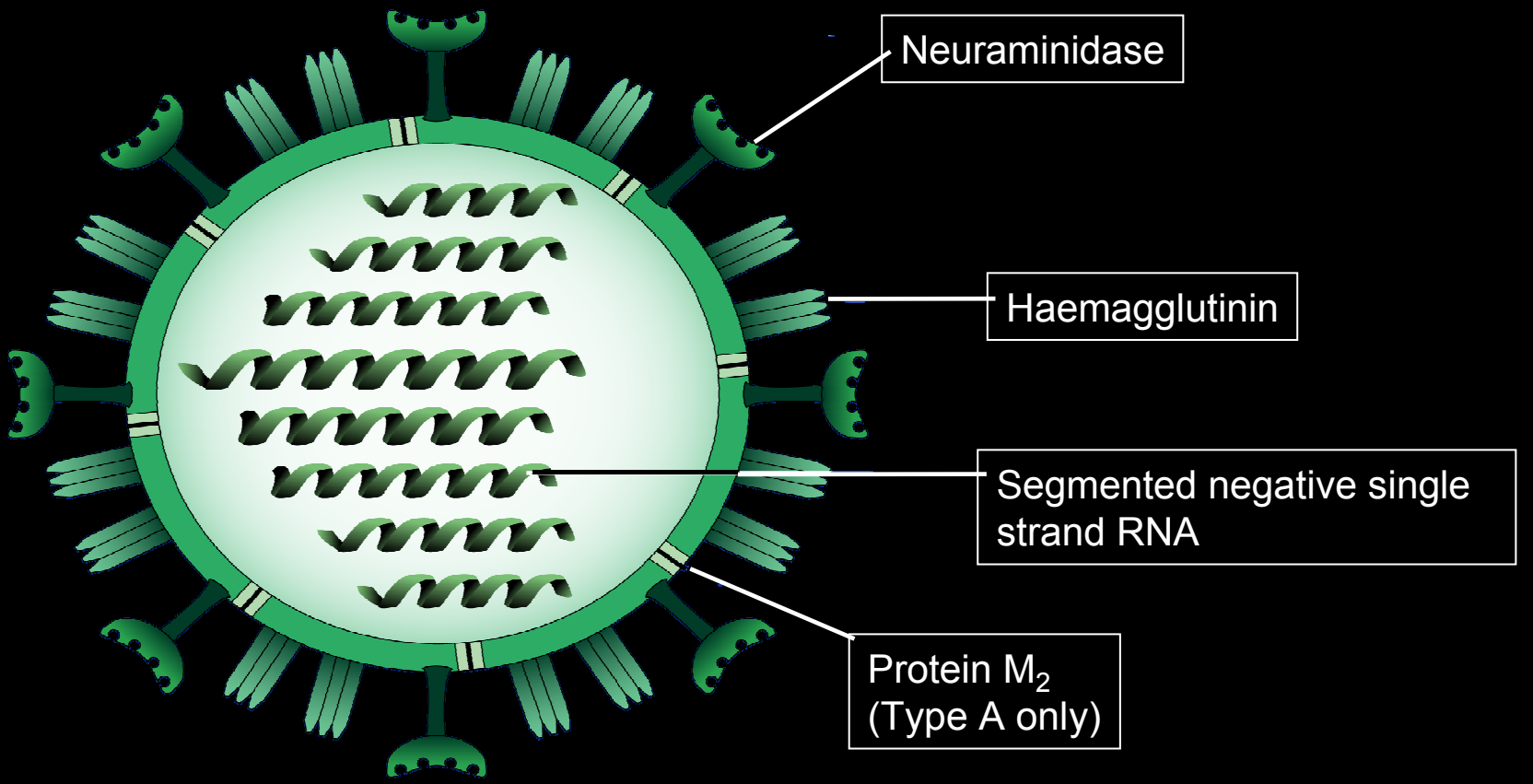
Schematic representation of influenza



Putative antiviral targets

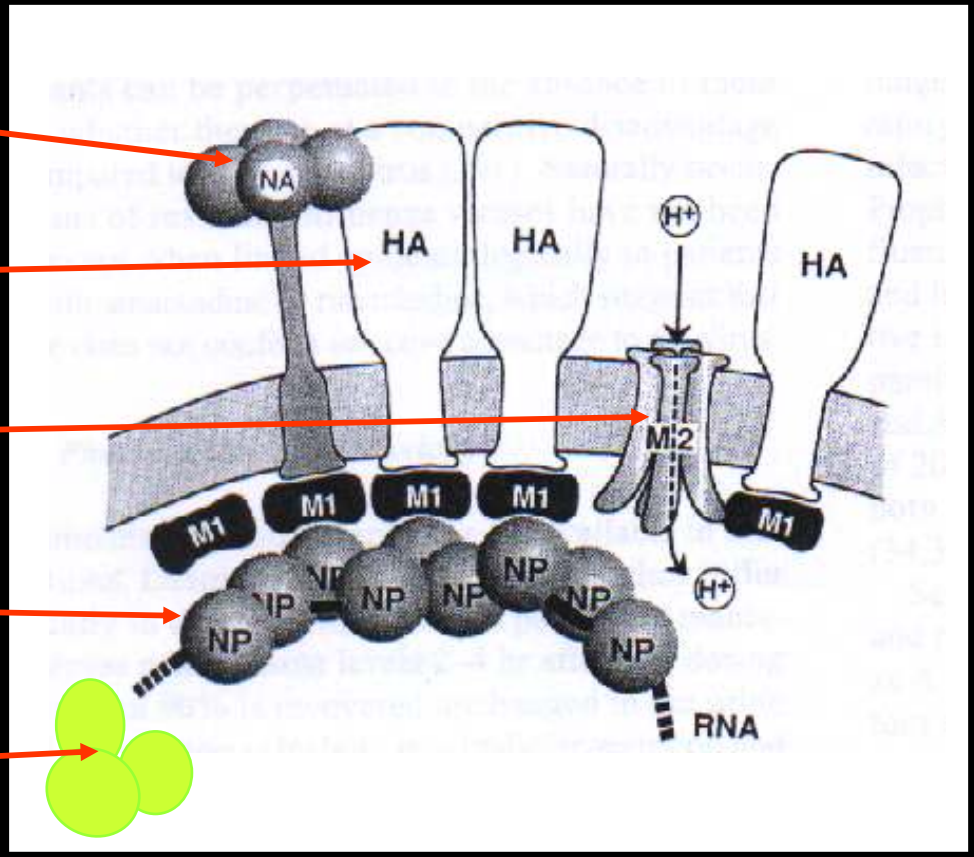
Neuraminidase inhibitors

Haemagglutinin inhibitors

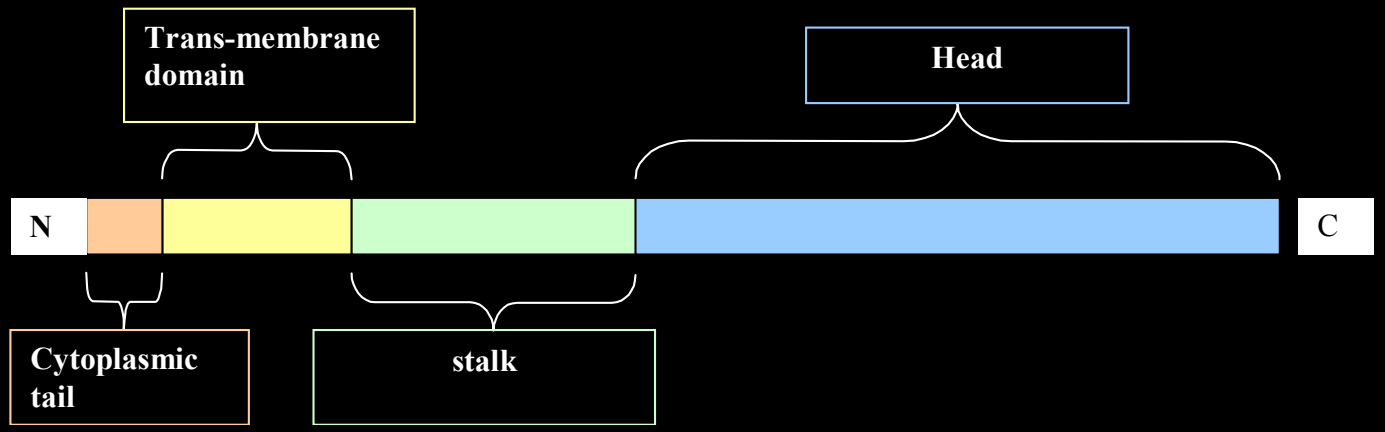
Ion channel (M2) blokers

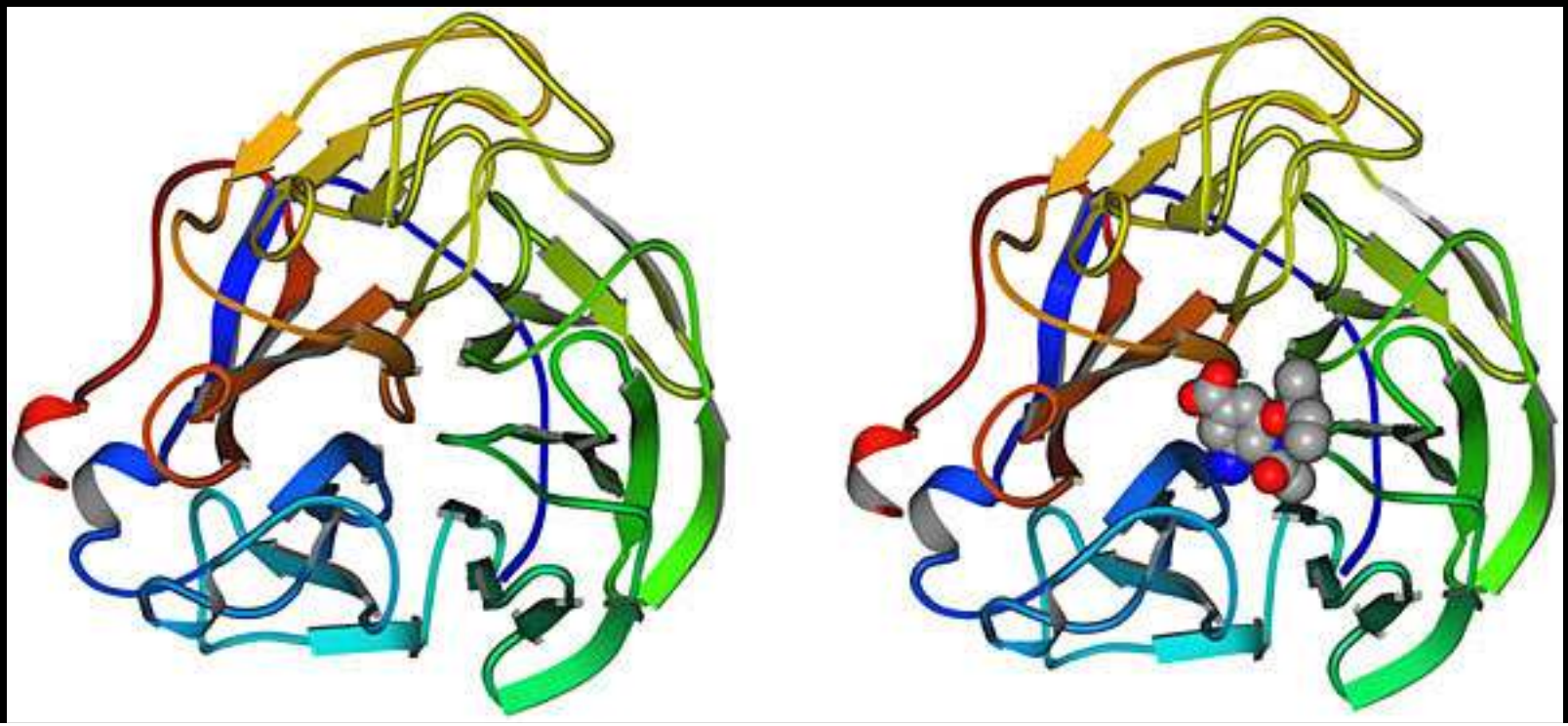
Nucleoprotein inhibitors

Polymerase inhibitors



Influenza Neuraminidase Glycoprotein



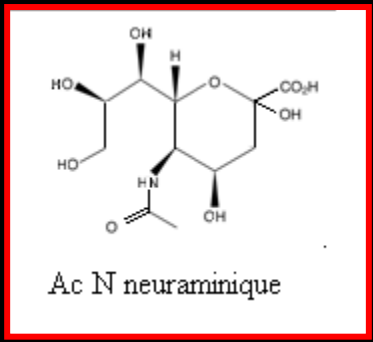


Commercially available influenza virus neuraminidase inhibitors

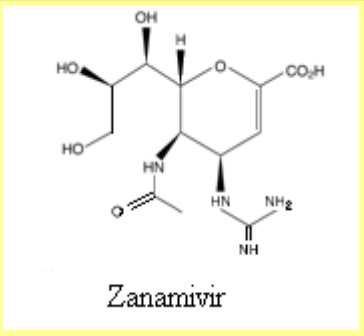
Zanamivir (Relenza®)

Oseltamivir (Tamiflu®)

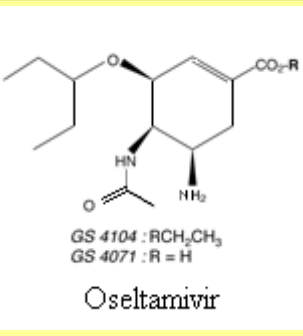
Peramivir (not shown)



Ac N neuraminique



Zanamivir



Oseltamivir

NAI Resistance : Preliminary data

Mutations associated with resistance and susceptibility to NAIs

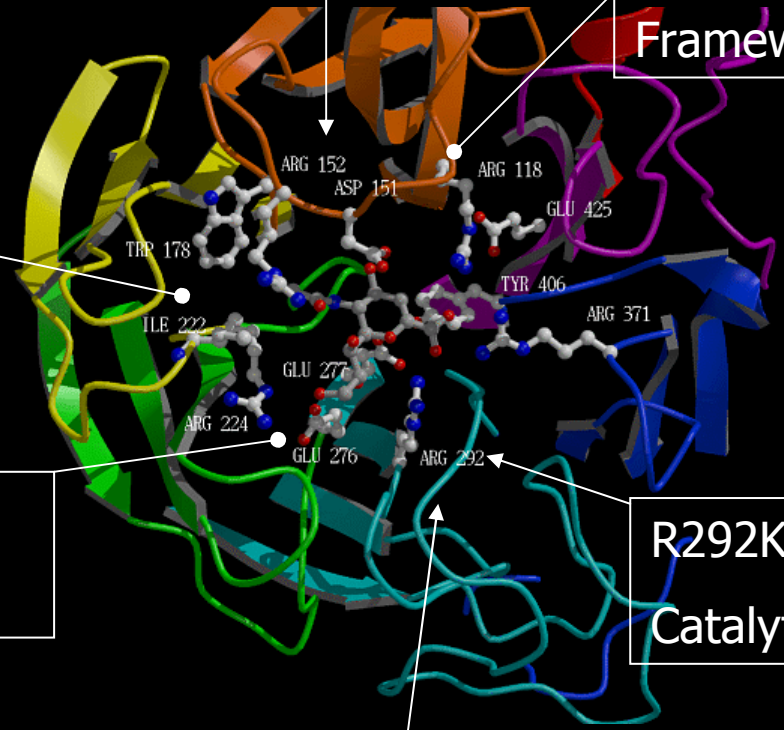
In vitro data from LV Gubavera, Vir Res 2004 :

function	position	virus	treatment	oseltamivir	zanamivir	peramivir
Framework	N119V	H3N2	Oseltamivir	R	S	S
	H274Y	H1N1	Oseltamivir	R	S	R
		H5N1	Oseltamivir	R	S	R
	D198N	B	Oseltamivir	R	S	S
Catalytic	R292K	H3N2	Oseltamivir	R	Nd/R	nd
	R152K	B	Zanamivir	R	R	R

Resistance to neuraminidase inhibitors in influenza A

R152K –
Catalytic mutation

E119V –
Framework mutation



D198N-
Framework mutation

H274Y-
Framework mutation

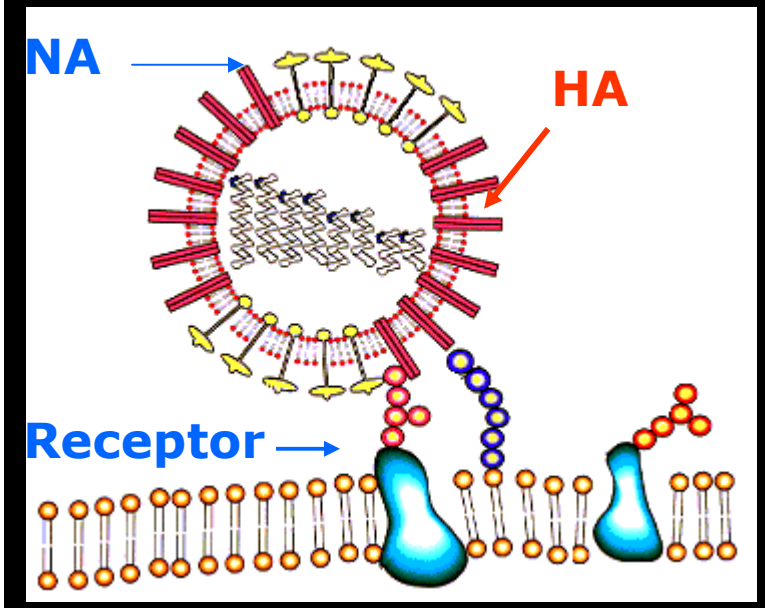
R292K-
Catalytic mutation

Asn294Ser-
Framework mutation ?

Mechanisms of Influenza virus resistance to neuraminidase inhibitors

Binding tight

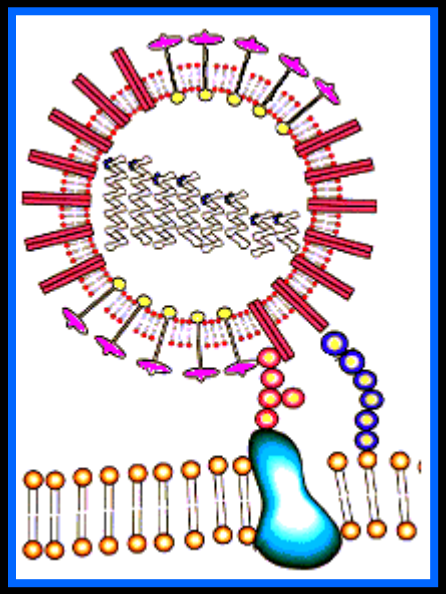
Release enhance (N2)



Fitness ++++

Binding Tight or moderate

NA dependence decrease

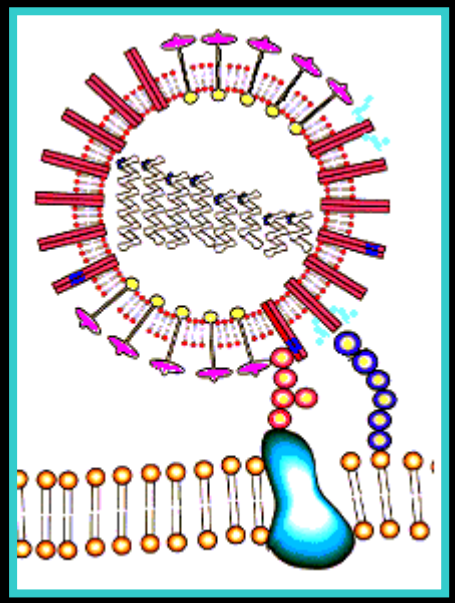


Resistant to inhibitor

Fitness +/-

Binding moderate

Release enhance (N2)



Decrease inhibitor efficiency

Fitness ++

A H5N1 and susceptibility to NAIs

Review about level of resistance

1- NISM (Antivir Res, 2005) : 0,4%

2- Kiso et al (Lancet, 2004) : 9/50 children

3- Ferraris et al (Antivir Res, 2005) : 0,9%

4- deJong et al (NEJM, 2005) : 2 cases of A H5N1

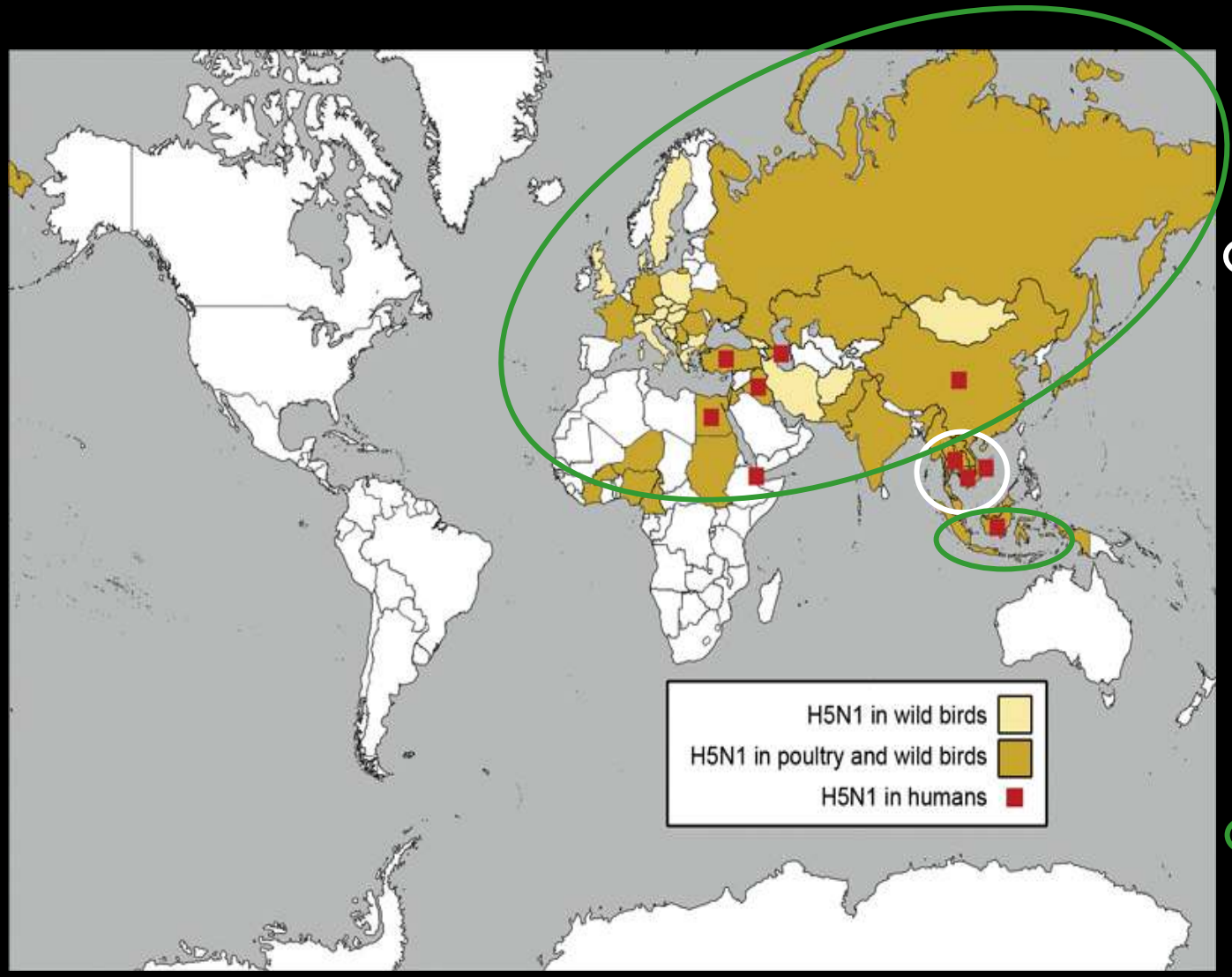
5- Whitley et al (Pediatr Infect Dis, 2001) : 4% in children

6- Evolution of the susceptibility of A H5N1 strains

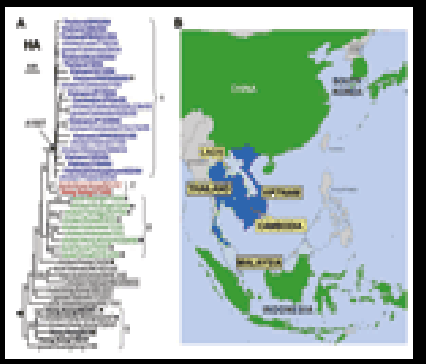
7- Ison et al (JID 2006) : analysis of 3 cases with resistant strains

8- Others...

Influenza A H5N1 as in July 2006

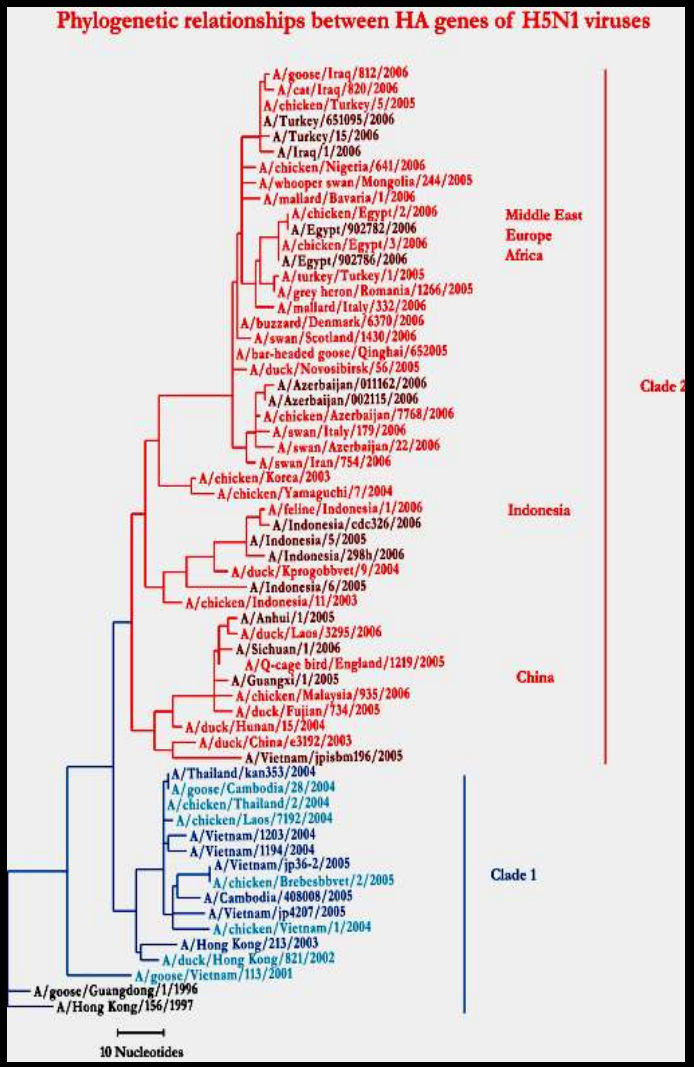


- Clade 1



- 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 poutry and humans are susceptible to both NAIs
- Viruses from the 2 clades may have different suceptibility 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 transmisible
- 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

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6- Evolution of the susceptibility of A H5N1 strains

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No concensus on what is the cut-off (threshold) for resistance

Impact of resistance on virus epidemiology

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 blokers

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 ?

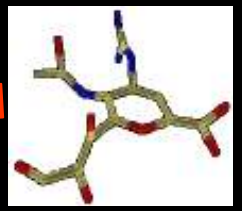
8- Different level of resistance depending on Na sub-type ?

Surveillance of emerging NAI-resistant H1N1 & H3N2 viruses

Fluorometric Neuraminidase Assay

❖Neuraminidase activity assay

❖Neuraminidase activity inhibition assay



Zanamivir
or
Oseltamivir

15 min – 37°C

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

1 H – 37°C

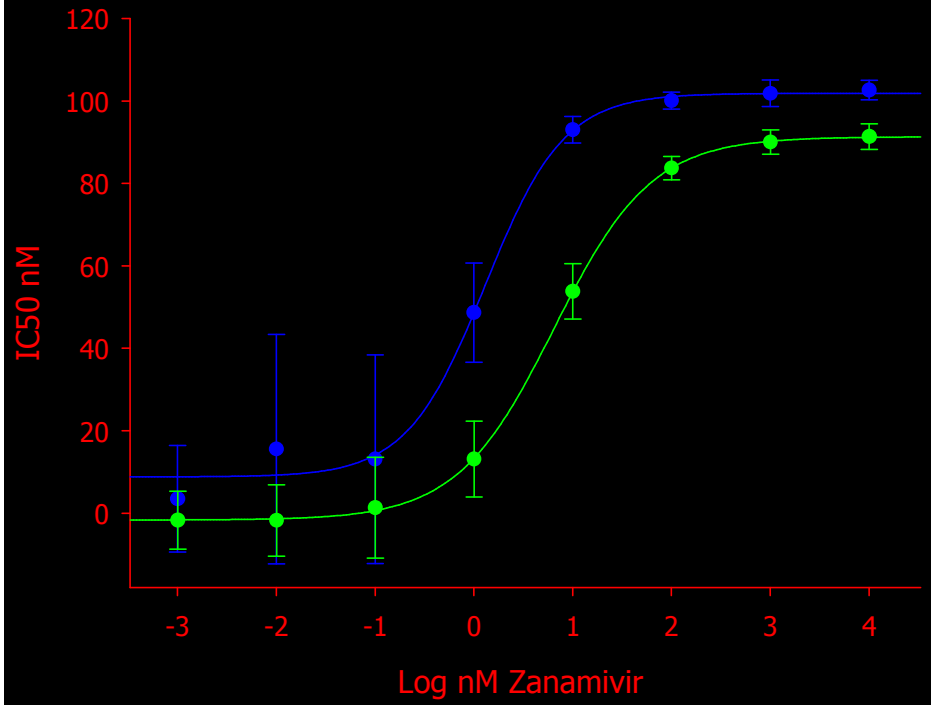
1 H – 37°C

Ex : 355nm

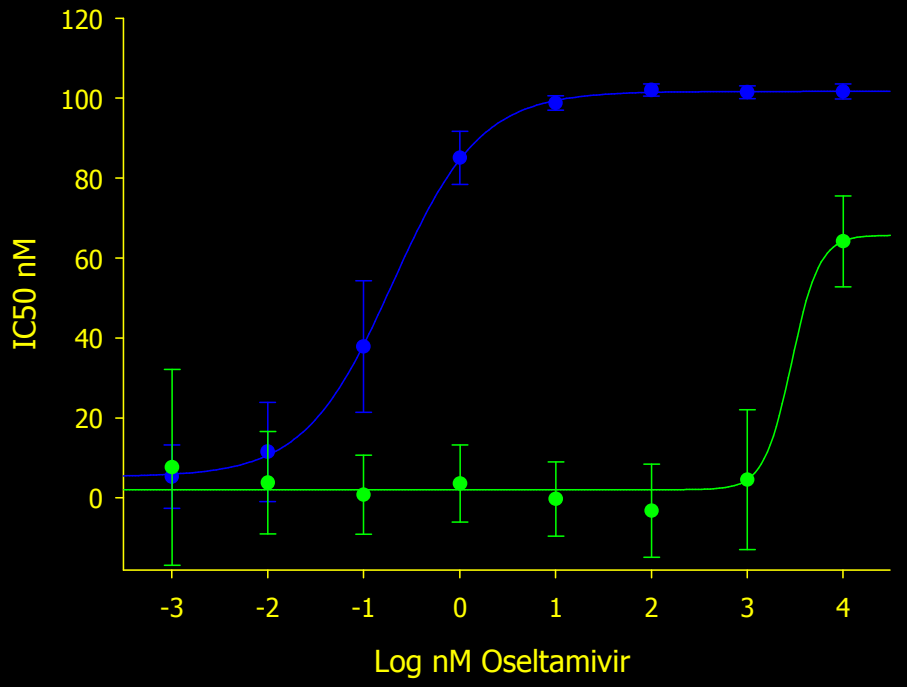
Em : 460 nm

R292K NAI profile

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



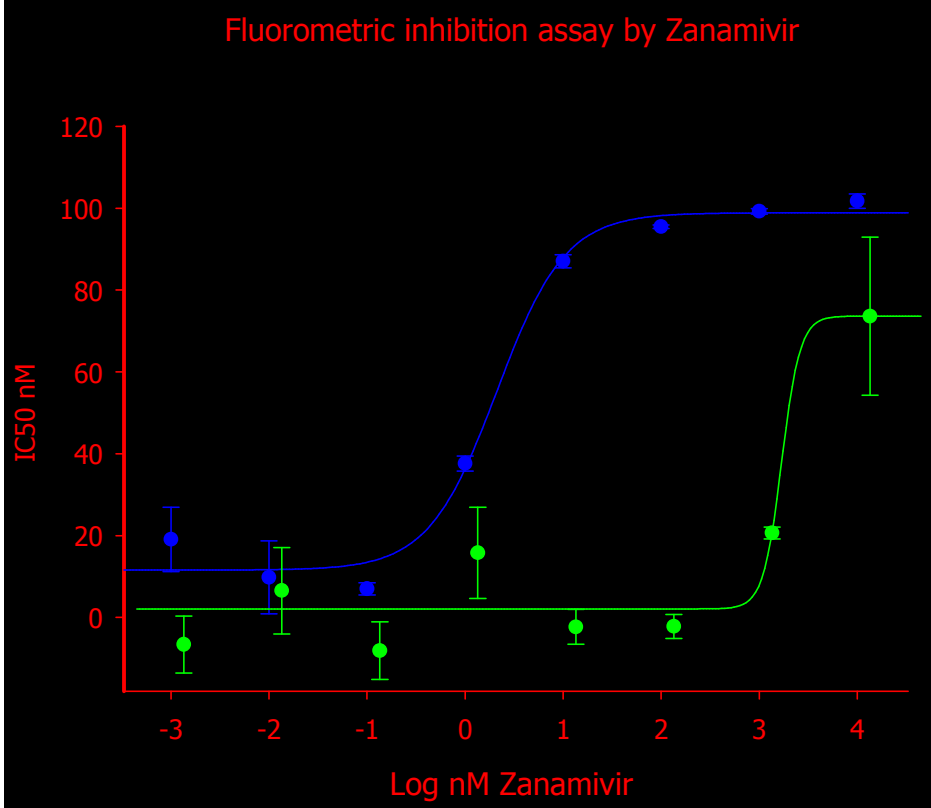
Neuraminidase inhibition fluorescence test on wild type virus and R292K by **Oseltamivir**



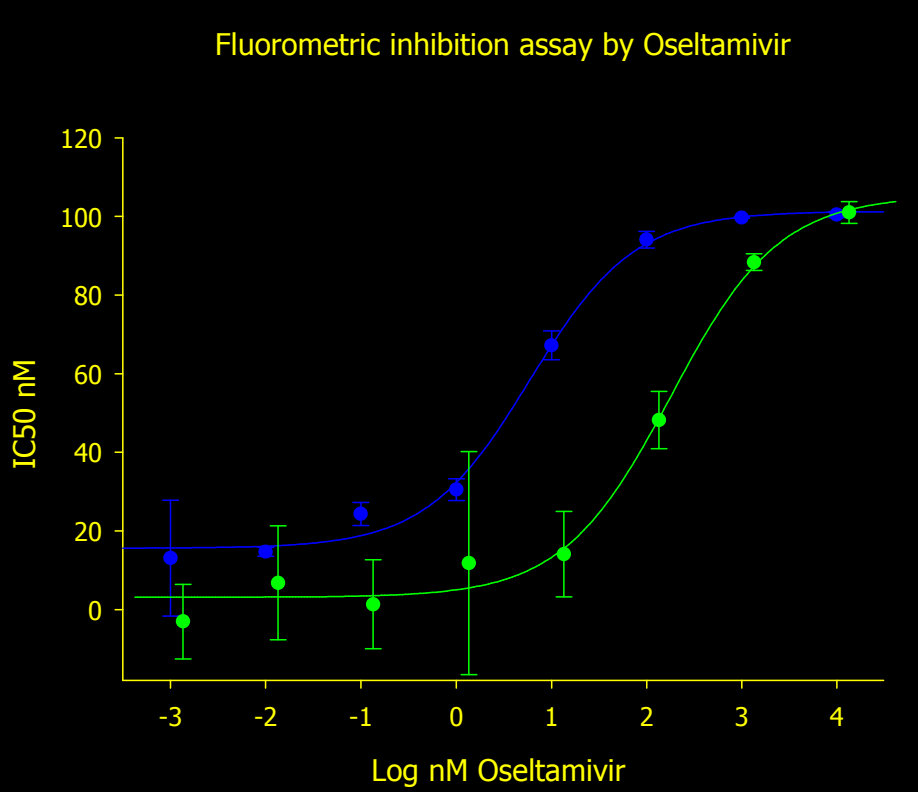
IC50 nM	Zanamivir				Oseltamivir			
	N	Mean	SD	CV	N	Mean	SD	CV
A/Sydney/05/97	28	3.7	1.8	48.7	28	0.67	0.37	55.3
R292K	28	22.86	7.66	33.5	28	14914	8728	58.5

E119V NAI profile

Neuraminidase inhibition fluorescence test on B/Beijing/1/87
Wild type and R152K mutant virus by **Zanamivir**



Neuraminidase inhibition fluorescence test on B/Beijing/1/87
Wild type and R152Kmutant virus by **Oseltamivir**

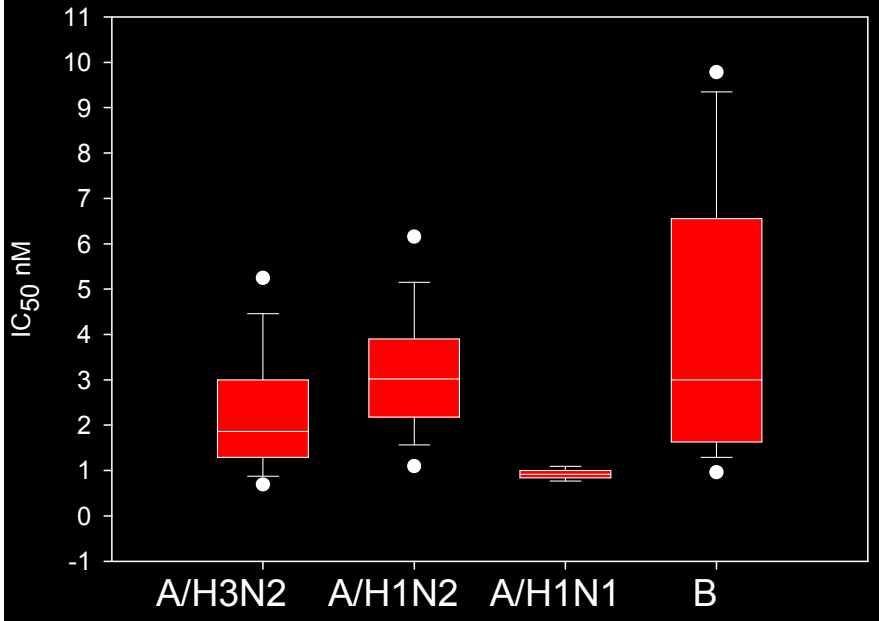


IC50 nM		Zanamivir				Oseltamivir		
	N	Mean	SD	CV	N	Mean	SD	CV
B/Beijing/1/87 WT	5	6.7	2.32	34.95	5	15.6	5.15	33.1
B/Beijing/1/87 R	5	6274	5363	85.5	5	438.2	83.1	19

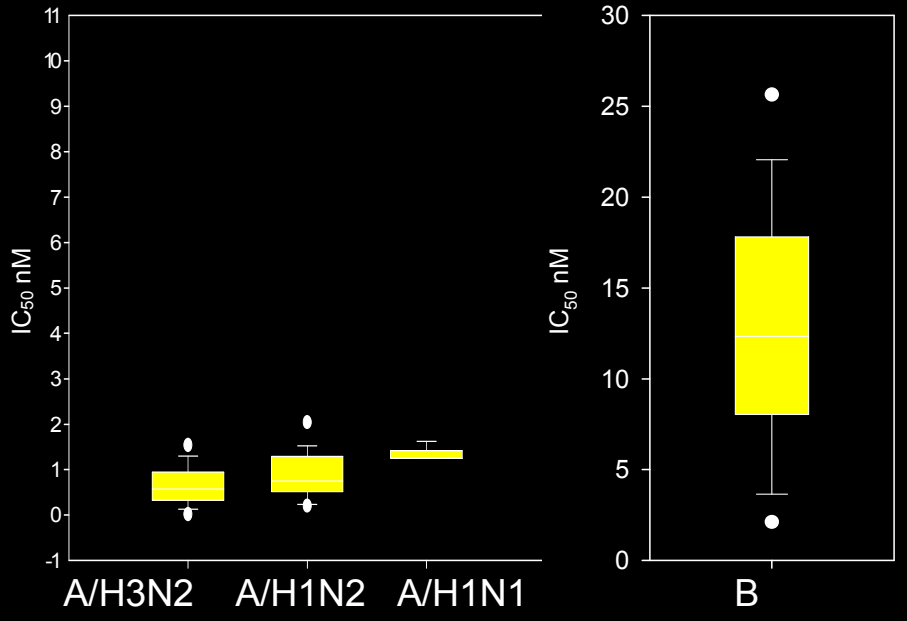
Sensitivity of influenza viruses to zanamivir and oseltamivir

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



IC50 Oseltamivir



Subtype	A/H3N2	A/H1N2	A/H1N1	B
Zanamivir (IC_{50} (nM))				
Mean	2.28	3.09	0.92	4.19
Max	5.97	6.70	1.09	11.90
Upper limit	**5.6**	**6.4**	**1.2**	**13.4**
Oseltamivir (IC_{50} (nM))				
Mean	0.67	0.9	1.34	12.99
Max	4.46	2.38	1.62	30.20
Upper limit	**2.1**	**2.2**	**1.7**	**33.5**

Susceptibility values

N2: Zanamivir < Oseltamivir

N1: Oseltamivir < Zanamivir

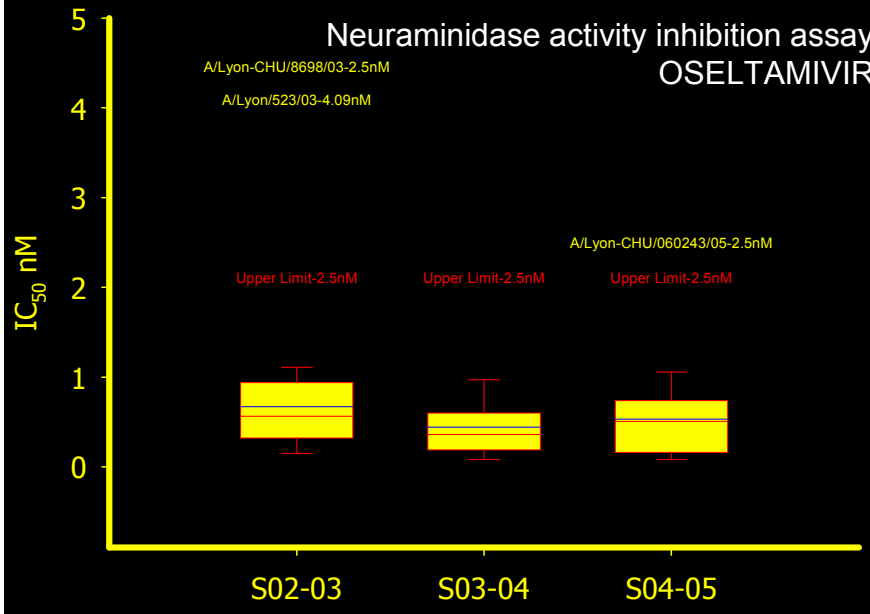
B

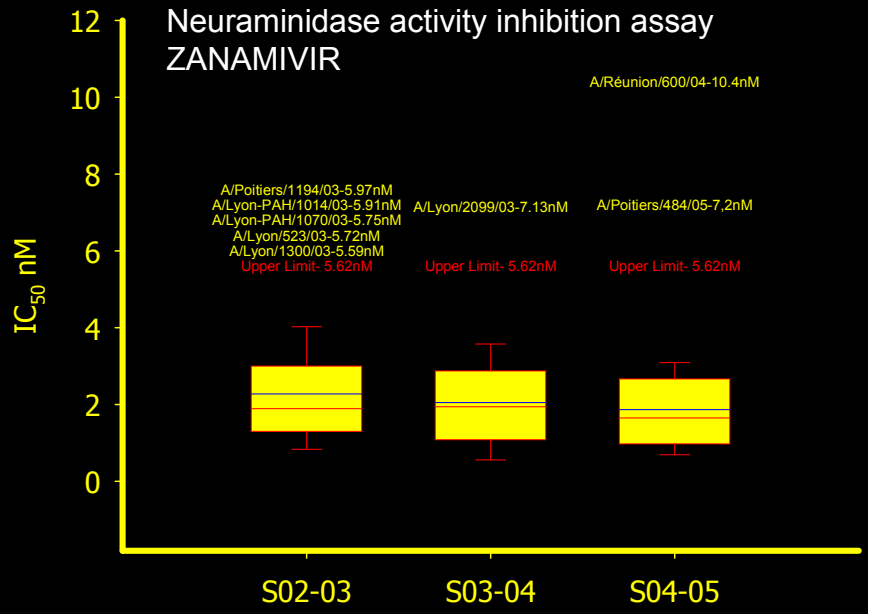
Detection of influenza virus with decreased suceptibility to neuraminidase inhibitors

1- Outliers viruses :

2- Resistant viruses :

Sensitivity of A/H3N2 influenza viruses to zanamivir and oseltamivir : 2002-2005



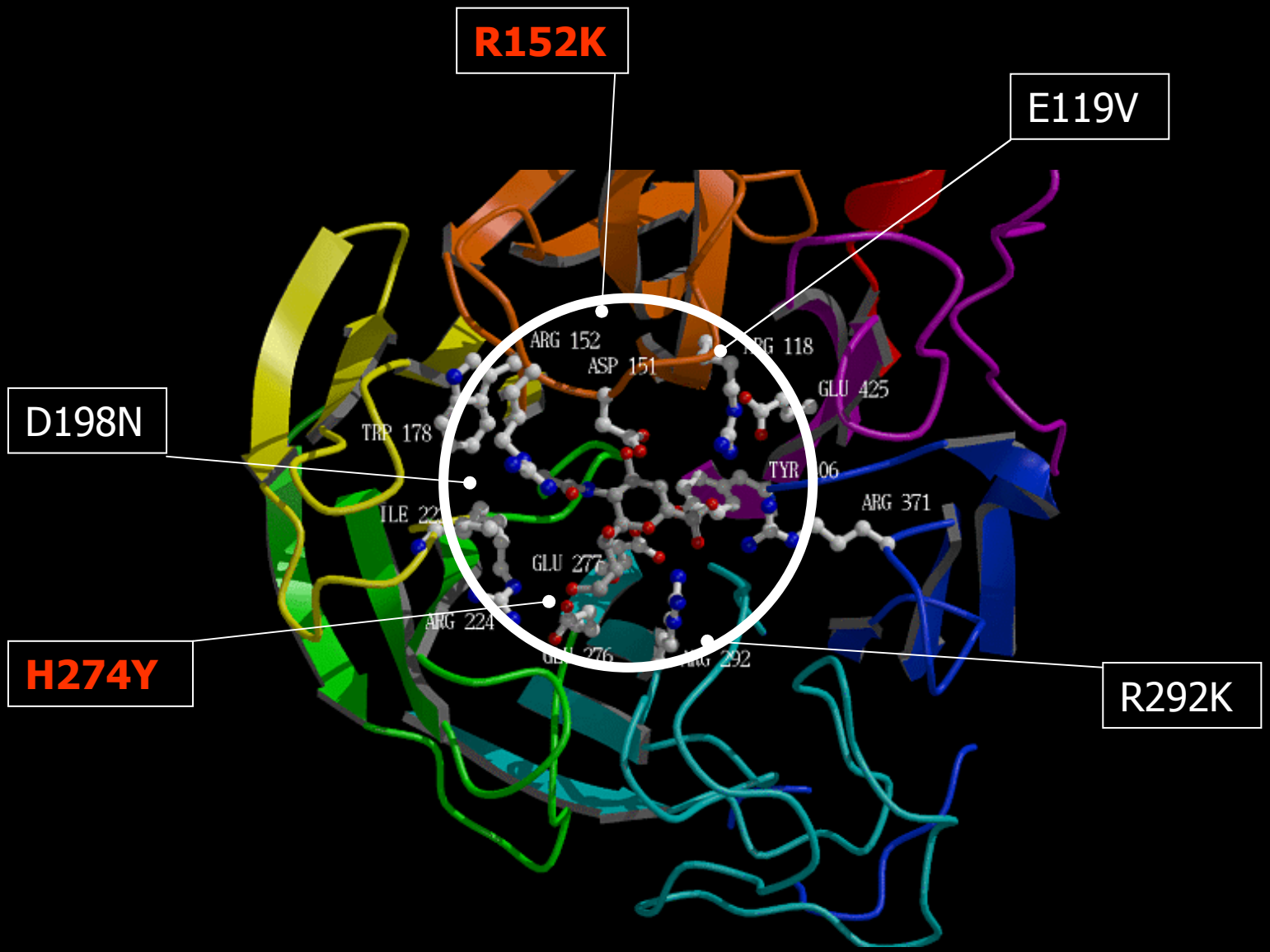


- H3 strains were tested with a fluorometric neuraminidase inhibition assay, underlining differences in IC_{50} range according to the anti-neuraminidase drug
- 3 viruses were isolated with upper limit oseltamivir IC_{50} values
- 8 viruses were isolated with upper limit zanamivir IC_{50} values
- 7 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



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