Programme influenza aviaire ARDIGRIP

New therapeutic and vaccine strategies against avian influenza

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Regards Croisés sur l'influenza aviaire

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CIRAD Lavalette, Montpellier

Teams

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- Francisco VEAS, U178 IRD, Montpellier; ImmunoClin Ltd, Paris
- Partners « South »

  S. Badur, Turkey; M. Ali, Egypt; R. Ndjooum, Cameroon; M. Dosso, Ivory-oast; Lopez-Lastra, Chili

Objectives

- Development of new therapeutic approaches
- Development of new vaccine approaches
- Development of tools
  - serology : sensitivity to neutralizing antibodies and cross-reactivity
  - sensitivity to inhibitors targeting the virus surface proteins

High through-put

Biosecurity

Sensitive

Quantitative

Transferable

Therapeutic approaches

SPECIFIC

antivirals

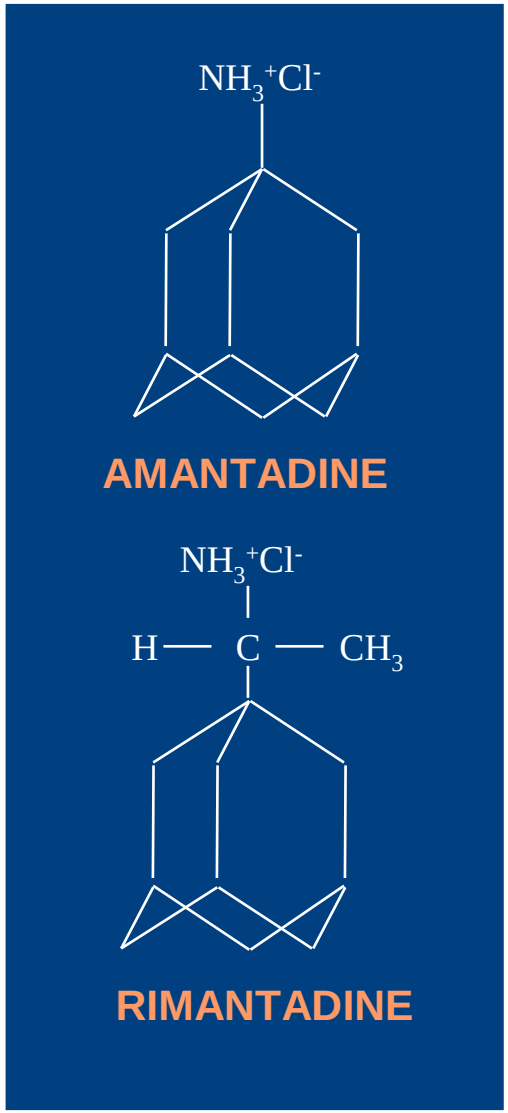
immunotherapy

NON SPECIFIC

innate responses

Adamantanes

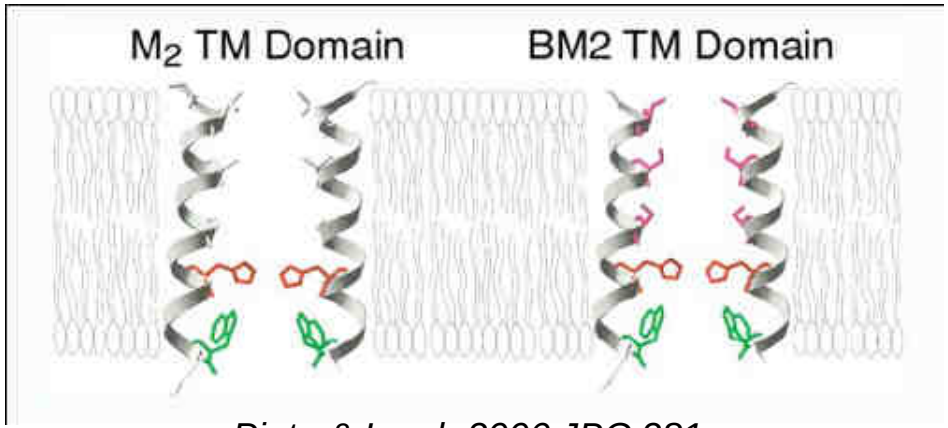
Amantadine and Rimantadine



M2 blockers

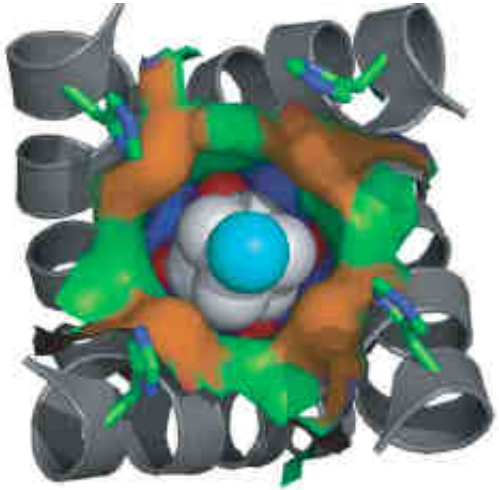
Target the M2 of influenza A viruses

NOT active against influenza B viruses



Pinto & Lamb 2006 JBC 281

Resistance to Adamantanes



Stouffer et al. 2008 Nature 451

	M2 aa residue				
	26	**27**	**30**	**31**	**34**
S	Leu	Val/Ile	Ala	Ser	Gly
R	Phe *His*	Ala Thr Ser	Val Thr Ser	Asn	*Glu*

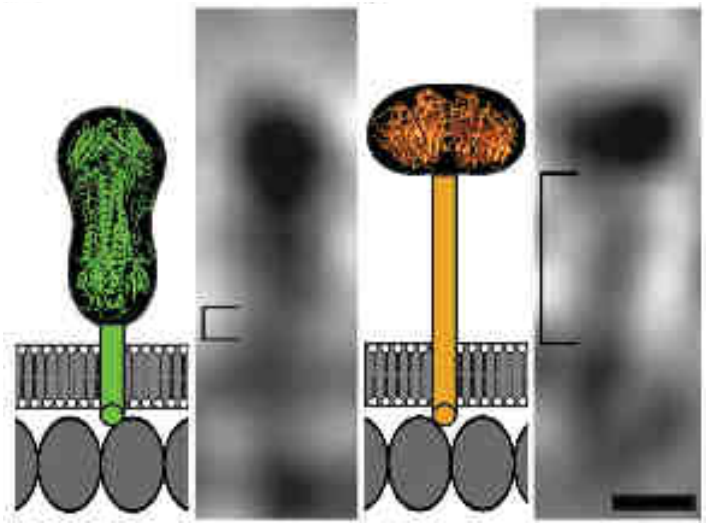
Resistance of H5N1 viruses

clade 1 and clade 2.1 resistant
clade 2.2 and 2.3 sensitive

No alteration of virus fitness

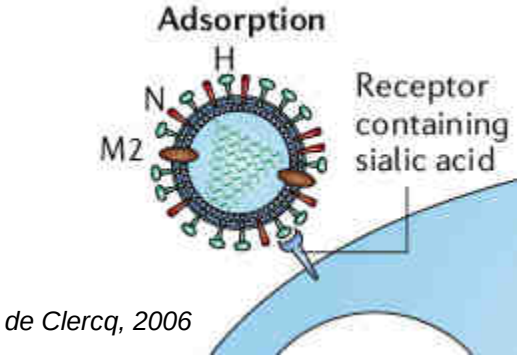
Anti-neuraminidase inhibitors

HA
Receptor binding
Attachment

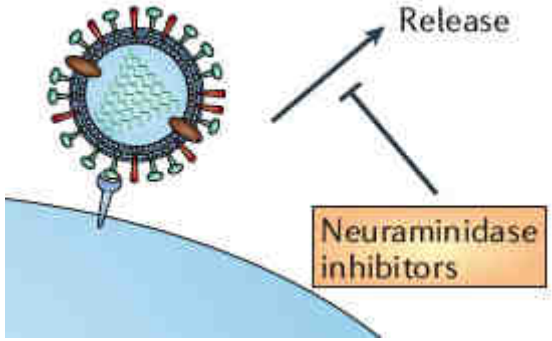


Harris et al, 2006

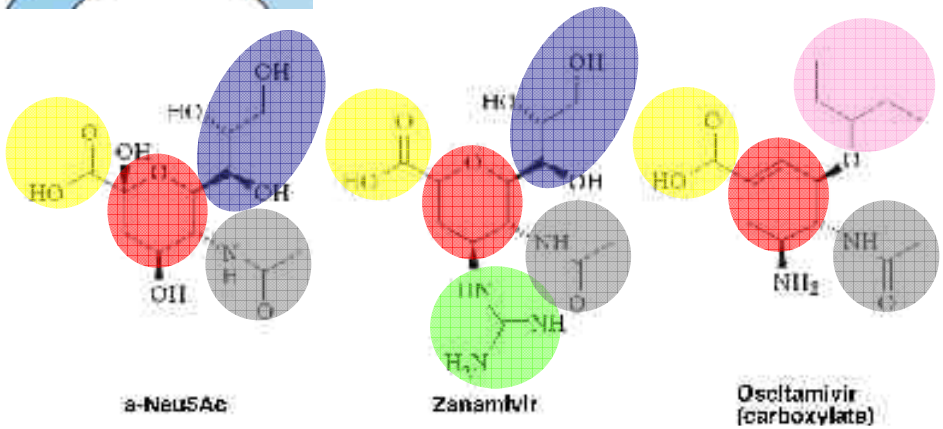
NA
cleavage of receptor
Virus Diffusion



de Clercq, 2006



Oseltamivir (Tamiflu®)
Zanamivir (Relenza®)



Selective for influenza
inhibit influenza A and B
Minor adverse effects

Resistance to anti-neuraminidase inhibitors

Seasonal influenza viruses

•Natural Resistance low (0-<1%) until 2007

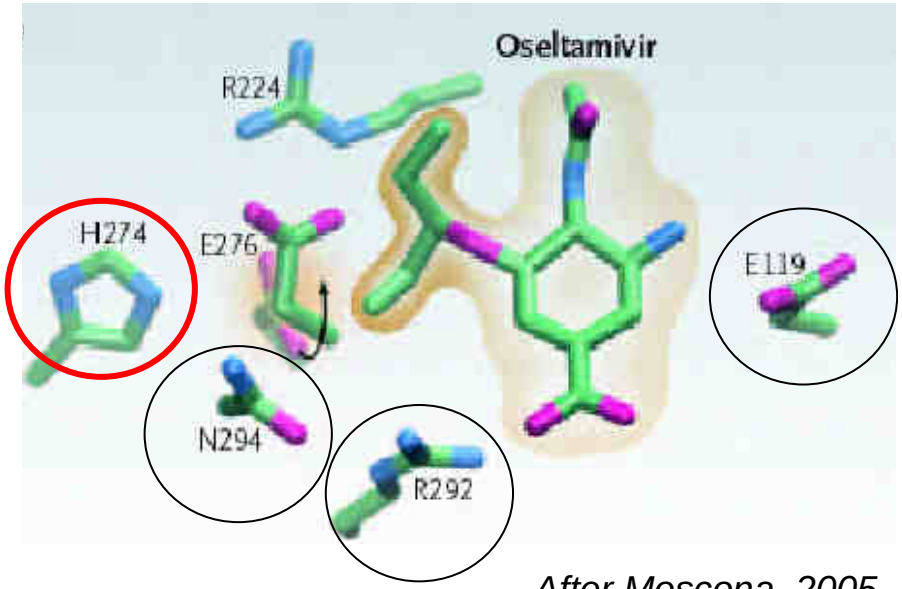
NISM (Antivir Res, 2005); Ferraris et al (Antivir Res, 2005);

•Emergence of resistant H1N1 viruses in 2007/2008

Lackenby et al (Eurosurveillance 2008)

•Resistance following treatment : higher in children (4-18%)

Ison et al (JID 2006); Whitley et al (Pediatr Infect Dis, 2001); Kiso et al (Lancet, 2004)



After Moscona, 2005

•N1 sub-type H274Y mutation

• sensitivity to zanamivir

H5N1 Viruses

•Resistance following treatment

deJong et al (NEJM, 2005)

•Variations in natural sensitivity

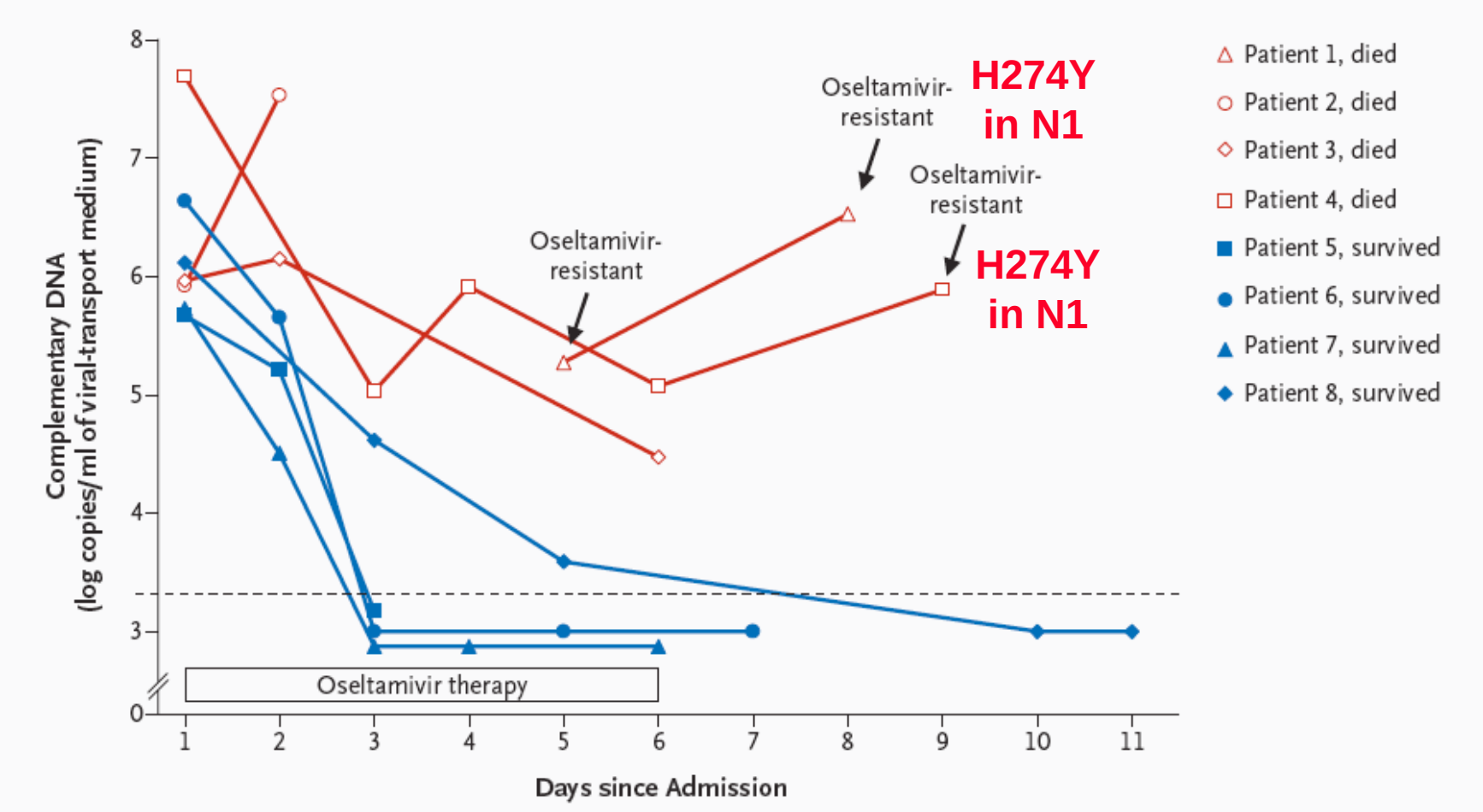
Increased : clade 1 viruses

Reduced : clade 2 viruses (Egypt N294S)

Rameix-Welti et al. 2006 AAC; McKimm-Breschkin et al. 2007 EID

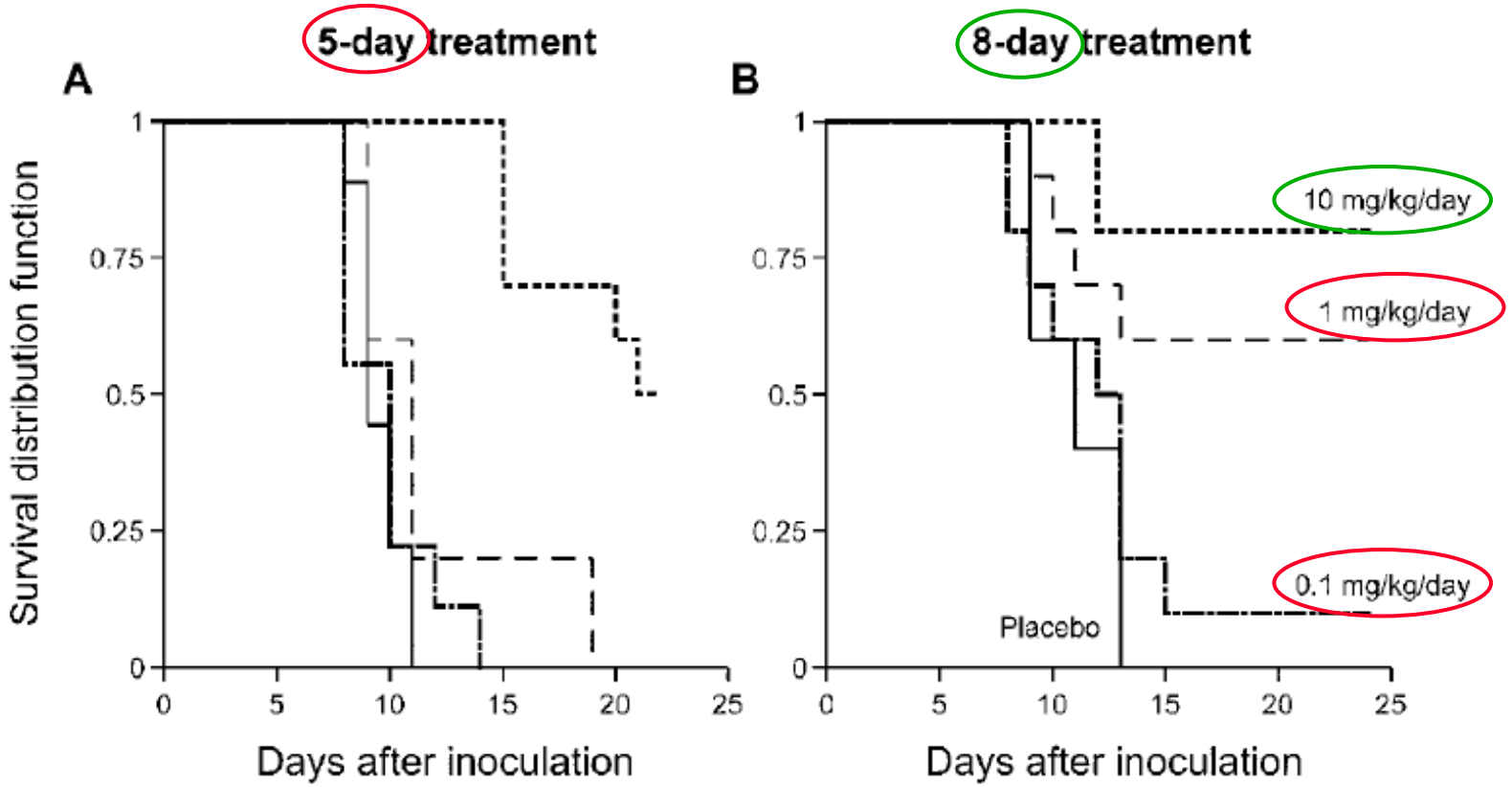
Effect on virus fitness?

Emergence of H5N1 resistant viruses



(de Jong et al, 2005)

Antivirals and H5N1 influenza viruses
Dose, Duration of treatment ?



Survival of BALB/c mice inoculated with 5 MLD_{50} of VN 1203/04 (H5N1) virus

(Yen et al, 2005)

Therapeutic approaches

SPECIFIC

antivirals

immunotherapy

NON SPECIFIC

innate responses

Human monoclonal antibodies

Vietnam04

Virus neutralization

		H3N2 Strain	H5N1 Strains[a]				
		Cal/7/2004	HK/491/97	HK/213/03	VN/1203/04	JPHN/30321/05	Indo/5/05
Sheep antisera[b]	Not known	<10	2,032	2,560	806	1,613	806
FLA5.10	1 mg/ml	<10	127	4,064	508	806	<10
FLA3.14	1 mg/ml	<10	403	508	226	508	508
FLD20.19	1 mg/ml	<10	905	5,120	1,613	6,451	5,120
FLD21.140	1 mg/ml	<10	32	>14,882	5,120	12,902	<10
A146[c]	0.31 mg/ml	<10	<10	<10	<10	<10	<10

Pos

Neg

Simmons et al. PLOS Medicine 2007

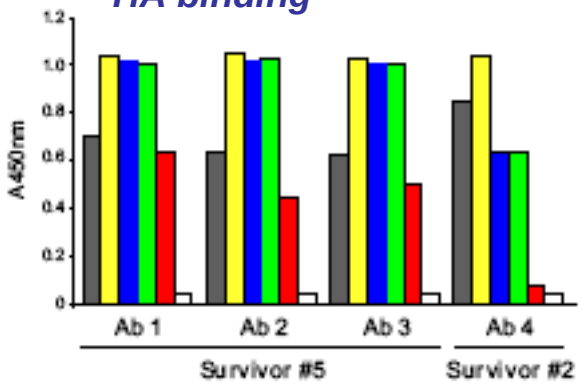
Turkey06

Virus neutralization

	H5					H1	H3
Antibody	A/Vietnam/ 1203/04*	A/Vietnam/ 1203/04*	A/Indonesia/ 5/05	A/Turkey/ 65596/06	A/Egypt/ 14725/06	A/New Caledonia/ 20/99	A/Hong Kong/68
Ab 1†	11–21	2.3–9.3	9.3	9.3	1.2–2.3	9	>333
Ab 2†	63	54–217	27	108	7–13	54–108	>333
Ab 3†	58	18	16	31	4–8	8–16	>333
Ab 4†	1.7–6.3	0.5–2.2	>333	Not done	Not done	>333	>333

Kashyap et al. 2008 PNAS 105

HA binding



H5-VN04
H5-TK06
H5-IND05
H1-NC99
H1-SC18
H3-WIS05

Post-infection therapy with anti-H5N1 monoclonal antibodies

VN04

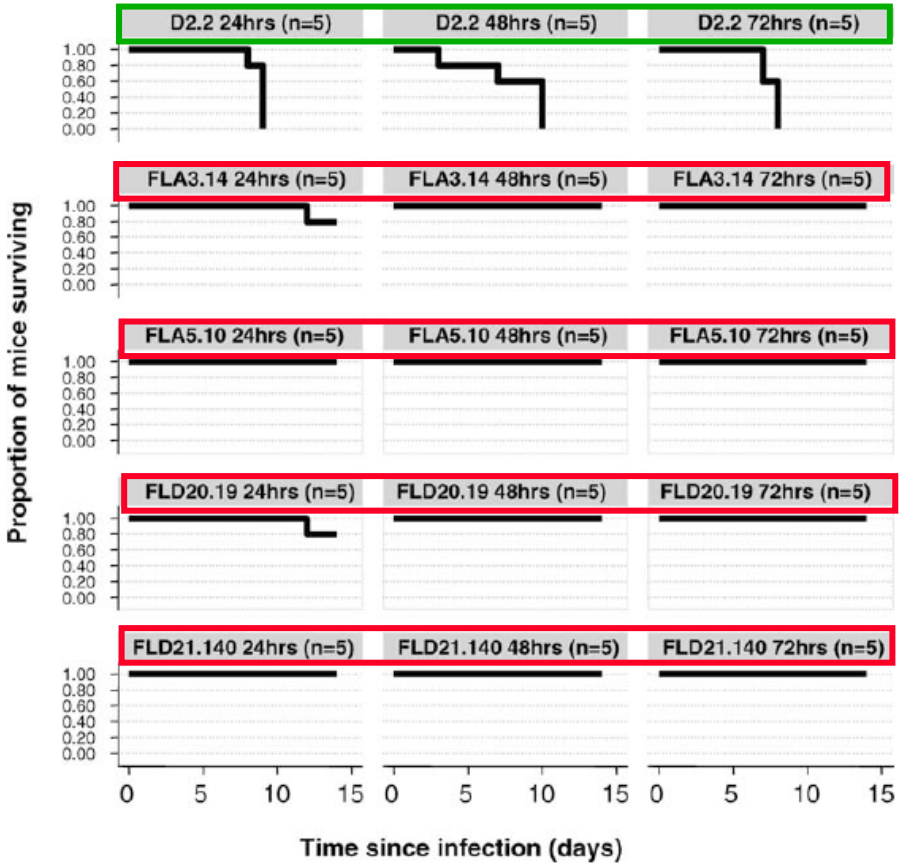


Figure 4. mAb Therapy and Survival in Mice with Established A/Vietnam/1203/04 (H5N1) Infection

Simmons et al. PLOS Medicine 2007

INDO5

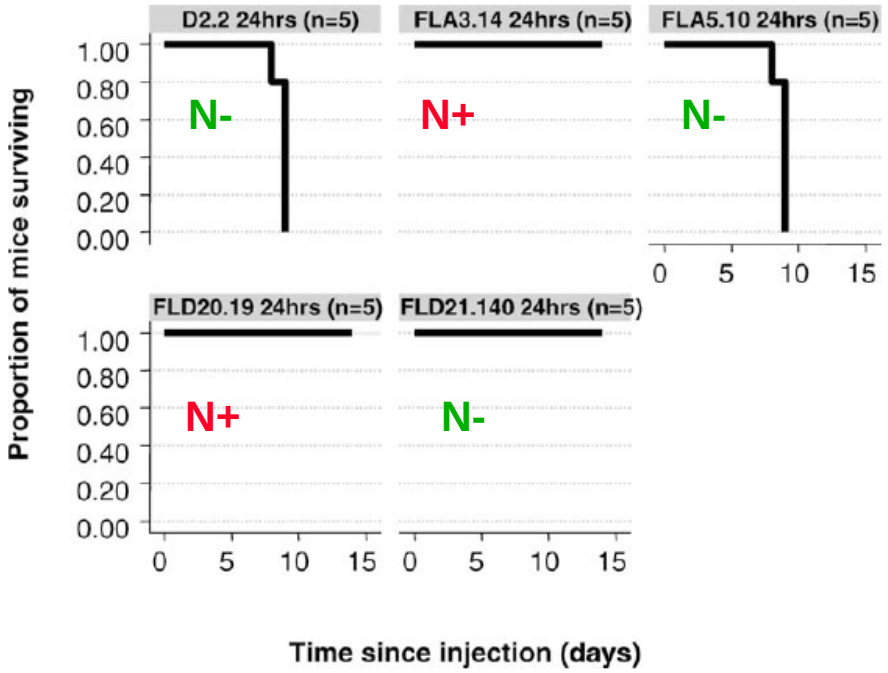


Figure 5. mAb Therapy and Survival in Mice with Established A/Indonesia/5/2005 (H5N1) Infection

N+ : neutralizing in vitro

N- : non neutralizing in vitro

Treatment with convalescent plasma

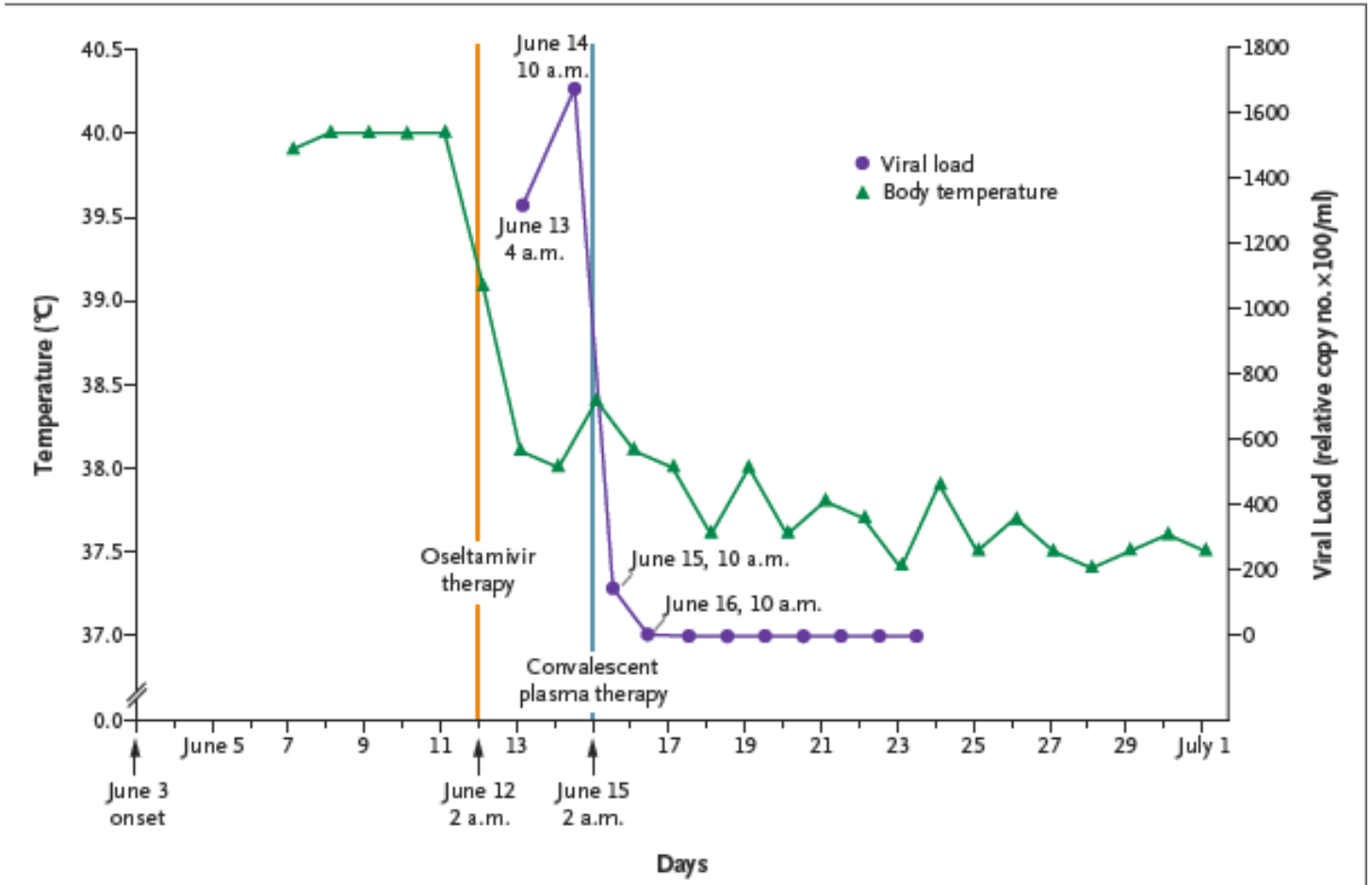


Figure 1. Influenza A (H5N1) Viral RNA Load in Tracheal Aspirates and the Patient's Response to Treatment.
The green line represents the patient's body temperature, and the purple line represents the viral load. The orange line represents the beginning of oseltamivir therapy, and the blue line represents the beginning of convalescent plasma therapy.

Zhou et al. NEJM 2007 357:1450

Human anti-H5N1 monoclonal antibodies

- Human antibodies obtained by **immortalization of B-memory lymphocytes** of contact subjects.
  - Infected Subjects (Turkey, Egypt)
  - Vaccinated Subjects
- Human antibodies selected from **“naive” human antibody** phage libraries

- Test reactivity
  - Target Proteins
  - Neutralizing potential
  - Cross-reactivity

- Evaluation of therapeutic and prophylactic potential in mice and ferrets

G. Gorochov, S. van der Werf, B. Lina, « Sud »

Therapeutic approaches

SPECIFIC

antivirals

immunotherapy

NON SPECIFIC

innate responses

Role of microbicides of human and animal hosts

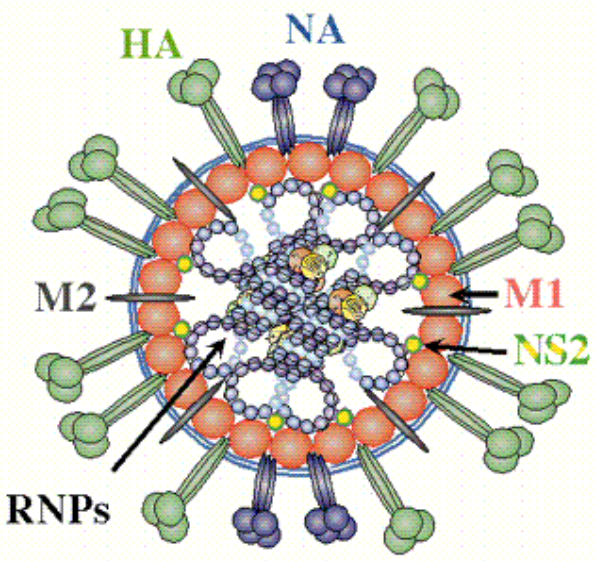
Natural Microbicides, innate response to viral infections

- Inductors of microbicides
- Cell lines and respiratory epithelial cells (bronchial, pulmonary) or enteric epithelium of human and avian hosts
- Evaluation of virus multiplication in vitro
  - Reference strains (H1N1, H3N2, B ; H5N1, other)
  - Therapeutic and prophylactic potential
  - Induction of cytokines/chemokines, mediators of inflammation
- Evaluation of therapeutic and prophylactic potential in vivo in mice and ferrets
- Other respiratory viruses?

F. Véas, ImmunoClin Ltd, S. van der Werf, Lopez-Lastra, Chili

Vaccine approaches

Development of tools



- Retroviral Pseudoparticles (PPV) axpressing virus surface proteins (HA, NA, M2)
  - various reference strains or primary isolates H1N1, H3N2, H5N1
  - Infectivity Tests
  - Fusion Test
  - HA-NA Balance
  - Receptor Specificity
  - Neutralization by antibodies
  - Inhibition by inhibitors targeting surface proteins

- Serological surveys (« South » partners) and technology transfer

B. Labrosse, F-L Cosset, S. van der Werf, B. Lina, « Sud »

Interactions