

Recent vaccine experience with novel antigens

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Recent vaccine experience with novel antigens - overview

- **Overview of five vaccine studies using novel haemagglutinins that have been conducted recently**
- **Consider the principal findings from each of the five studies**
- **Summarise the data, identifying points to consider for the design of a clinical protocol to evaluate a pandemic vaccine**

Published studies using H5 haemagglutinins - 1

First author	Antigen/ (formulation)	No. Subjects (Age)	No. groups	No. doses (time)	Dosage (μ g)
Treanor 2001	rH5 (Plain)	147 (?)	15	2 (d0, 21) (d0, 28) (d0, 42)	(25, 25) (45, 45) (90, 90) (90, 10) (0, 0)
Nicholson 2001	H5N3 (SA \pm MF59)	65 (18-40)	6	2 (d0, 21)	(7.5, 7.5) (15, 15) (30, 30)
Stephenson 2003	H5N3 (SA \pm MF59)	26 (18-40)	6	1 (16M)	(7.5) (15) (30)

Published studies using H5 haemagglutinins - 2

First author	Serology (days)	No. bleeds	Assessments
Treanor 2001	MN, EI (V1, V1+14) (V2, V2+: 7, 14, 21, 28)	7	Safety Dose response Effect of dose interval Kinetics MN titre \geq1:80
Nicholson 2001	HI, SRH, MN (V1, V1+21) (V2, V2+21)	3	Safety Dose response Adjuvant effect CPMP criteria
Stephenson 2003	HI, SRH, MN (V3, V3+21)	2	Safety Boosting effect Adjuvant effect CPMP criteria

Published studies using H9 & H2 haemagglutinins - 1

First author	Antigen/ (formulation)	No. Subjects (Age)	No. groups	No. doses (time)	Dosage (μ g)
Hehme 2001	H2N2 (WV + AIPO ₄)* H2N2 (SP)	196 (18-30)	4	2 (d0, 21)	(1.9, 3.8, 7.5) (15)
	H9N2 (WV + AIPO ₄)* H9N2 (WV)	194 (18-60)	4	2 (d0, 21)	(1.9, 3.8, 7.5) (15)
Stephenson 2003	H9N2 (WV, SA)	60 (18-60)	6	2 (d0, 21)	(7.5, 7.5) (15, 15) (30, 30)

*AIPO₄ : 0.5mg/dose

Published studies using H9 & H2 haemagglutinins - 2

First author	Serology (days)	No. bleeds	Assessments
Hehme 2001	HI (V1, V1+10) (V2, V2+21)	4	Safety CPMP criteria
Stephenson 2003	HI, MN (V1, V1+21) (V2, V2+21)	3	Safety Dose response WV vs SA CPMP criteria Age effect

Strategies for H5N1 vaccine development

- **Attenuate' the A/Hong Kong/97 virus by removing basic amino acids from cleavage site. Rescue HA & NA genes into suitable viruses by reverse genetics:**
 - **A/Ann Arbor/6/60, in USA**
 - **A/Duck/Hong Kong/836/80 (H3N1), in Japan**
- **'Express the H5 HA in baculoviruses by recombinant technology' (Treanor 2001)**
- **'Use a surrogate apathogenic H5N1 virus'.**
 - **A/Duck/Singapore-Q/F119-2/97 (NIB-40), whose HA was similar to that of the H5 strains. (Nicholson 2001)**
 - **R513, an H5N1 reassortant between A/Duck/Hokkaido/67/96 (H5N4) and A/Duck/Hong Kong/301/78 (H7N1)**

Phase I studies of baculovirus expressed avian H5 HA

- Katz J & Treanor J. “Vaccines and related biological products advisory committee meeting regarding influenza vaccine formulation for 1999-2000. 1999”

Phase I trial:

- Recombinant H5 HA administered as two 10 or 20 μ g doses to 56 subjects .
 - 2/28 receiving 10 μ g dose developed VN Ab \geq 1:80.
 - 6/28 receiving 20 μ g dose developed VN Ab \geq 1:80.

Phase II studies of baculovirus expressed avian H5 rHA (A/HK/156/97)

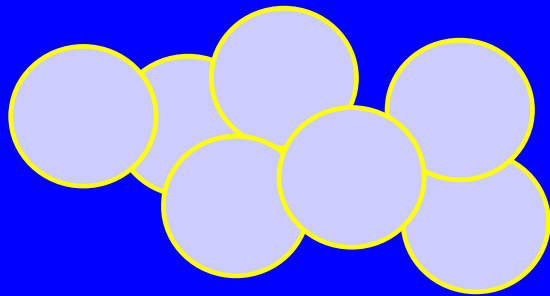
Dose 1/ Dose 2 (µg)	No. with 156 MN response* /No. tested when given vaccine at intervals of:				≥4-fold 483 EIA response
	21	28	42	Any (%)	
25/25	1/10	2/10	2/9	5/29 (17)	5/24 (21)
45/45	1/10	4/9	3/10	8/29 (28)	7/24 (29)
90/90	5/9	6/10	4/10	15/29 (52)	8/19 (42)
90/10	4/10	4/10	2/10	10/30 (33)	8/23 (35)
Any rH5	11/39	16/39	11/39	38/117 (32)	28/90 (31)
Placebo	1/9	0/9	0/8	1/26 (4)	0/20 (0)

- Only 1/58 (2%) of subjects in combined 25 & 45 µg groups achieved a ≥4-fold increase following a single dose, compared with 23% (14/60) of subjects given 90 µg ($p < 0.01$)
- Frequency of response to two doses dependant on the total dose of vaccine administered ($p = 0.04$)
- Little or no variation in response rate with interval between doses ($p = 0.38$)

*Titre (4 weeks after 2nd vaccination) of ≥1:80 in at least 2 independent assays & +(ve) in Western blot with purified HK/156 HA & serum dilute 1/100

A/Duck/Singapore/97 (H5N3) MF59 adjuvanted vaccine

MF 59 oil in water emulsion

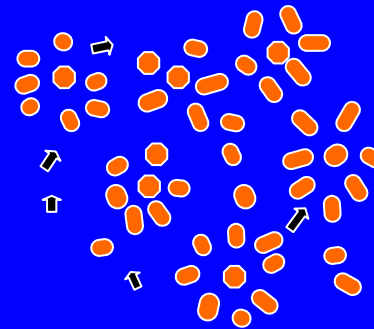


Oil phase: 9.75 mg Squalene
(cholesterol metabolite)

Water phase: 1.175mg Polysorbate 80
(Water soluble surfactant)

+

H5N3 surface antigen



+

1.175 mg sorbitan trioleate
(oil soluble surfactant)

+

water in citrate buffer

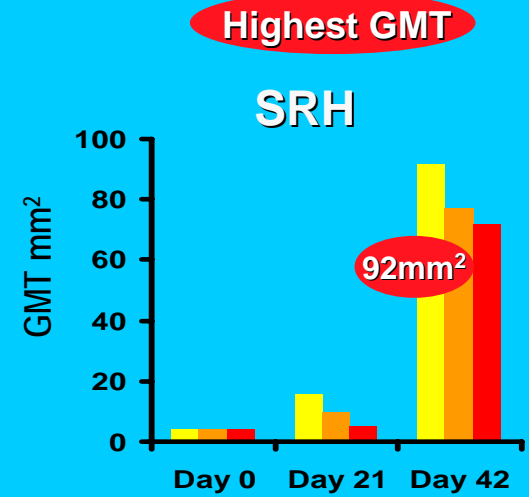
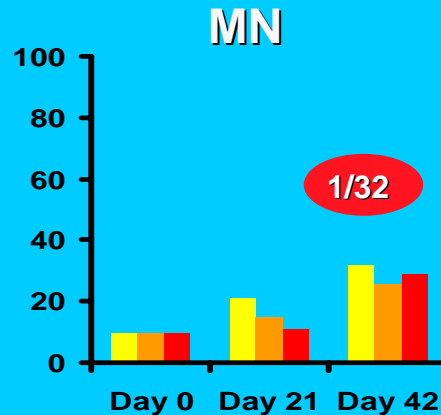
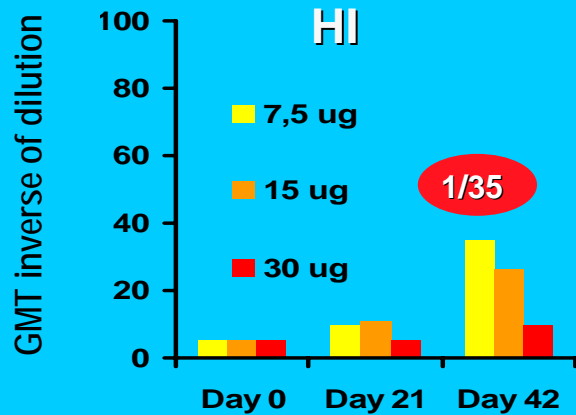
Local and systemic reactions to both injections

	Haemagglutinin content and type of vaccine						p
	7.5 µg		15 µg		30 µg		
	MF59 (n=10)	SA (n=11)	MF59 (n=11)	SA (n=11)	MF59 (n=11)	SA (n=11)	
Local							
Pain							
None	5	6	2	5	2	8	0.07
Mild	5	5	5	6	4	3	-
Moderate	0	0	3	0	4	0	-
Severe	0	0	1	0	1	0	0.0009†
Fever (≥38°C)	1	0	0	0	0	0	0.15
Erythema	0	0	0	0	0	1	1.0
Induration	0	0	0	0	0	0	1.0
Systemic							
Chills	1	2	0	0	0	1	0.40
Fatigue	2	1	1	3	3	1	0.72
Myalgia	1	3	4	0	2	1	0.25
Arthralgia	0	2	1	0	0	1	0.70
Headache	4	4	2	5	6	4	0.67
Nausea	1	1	0	0	2	2	0.58
Diarrhoea	0	0	1	2	1	0	0.70
Stayed at home due to reaction	1	0	0	0	0	1	0.42
Analgesia/antipyretic use	2	1	1	1	5	2	0.30

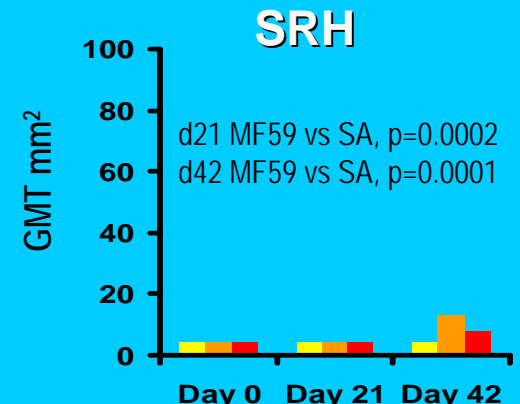
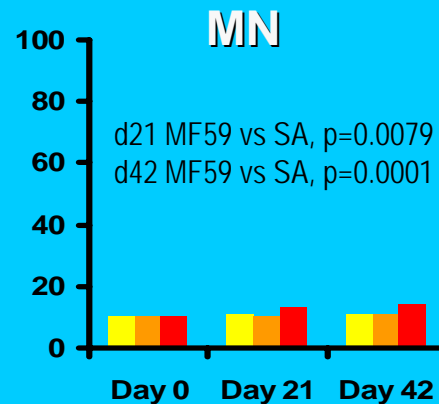
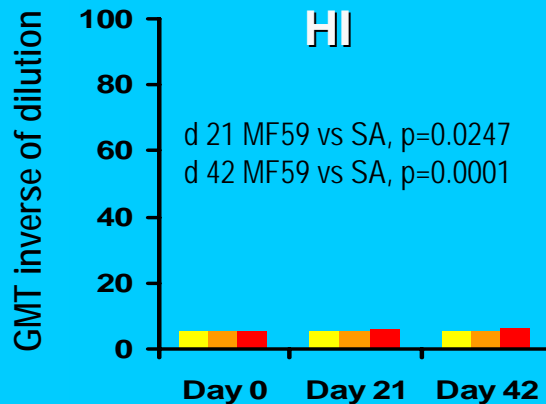
† Moderate & severe pain 9/32 (28%) vs 0/33

Geometric mean HI , MN, & SRH (H5N3) antibody responses

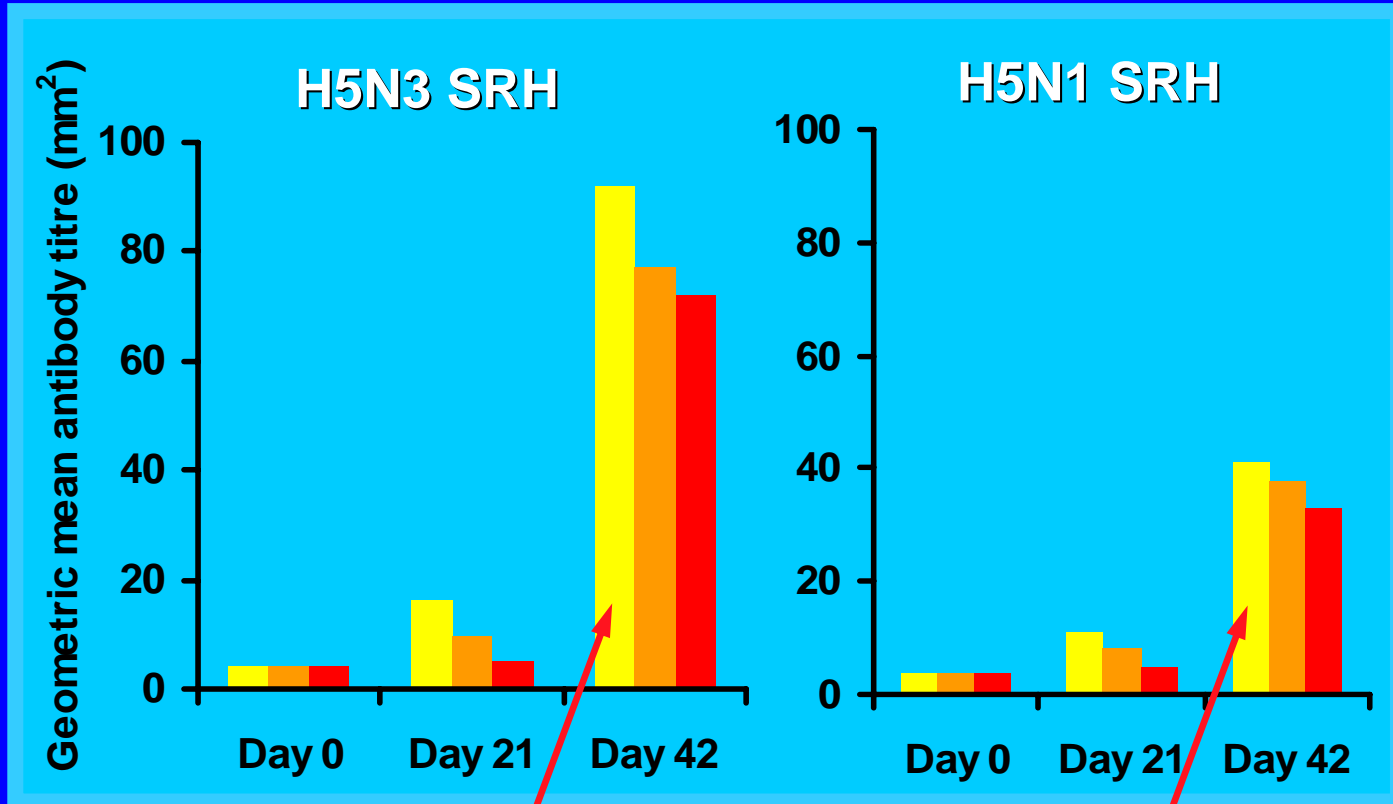
MF-59-adjuvanted



Non-adjuvanted



H5N3 & H5N1 SRH GMTs (mm²) to MF-59 adjuvanted vaccine



GMT 92mm²

GMT 41mm²

H5N3 vs H5N1 p<0.0001

SRH results (H5N1) in relation to CPMP criteria

Haemagglutinin content and type of vaccine

	7.5 µg		15 µg		30 µg	
	MF59	SA	MF59	SA	MF59	SA
	(n=10)	(n=11)	(n=11)	(n=11)	(n=11)	(n=11)
Day 21						
Mean GMT increase (>2.5)	2.79 †	1	1.99	1	1.16	1
% SRH titre >25mm ² (>70%)	40	0	0	0	0	0
% seroconversions (>40%)	40	0	0	0	0	0
Day 42						
Mean GMT increase (>2.5)	10 †	1	9.85 †	1.33	8.47 †	1.2
% SRH titre >25mm ² (>70%)	90 †	0	82 †	0	80 †	9
% seroconversions (>40%)	90 †	0	82 †	0	80 †	9

On day 21, none of the CPMP criteria were satisfied using the H5N3 HI test.

On day 42, 7.5 & 15µg formulations of MF59 vaccine satisfied 2 of 3 criteria using the H5N3 HI test

None of the non-adjuvanted vaccine formulations satisfied the CPMP criteria using the HI test requirements.

Boosting of immunity to influenza H5N1 with A/Duck/Singapore/97 vaccine

Aims

- **To assess durability of response and residual immunity at 16 months**
- **To assess effect of single H5N3 revaccination (MF59 or non-adjuvanted) on a primed immune system**

Response to A/Duck/Singapore/97 revaccination at 16 months - 1

**Study population: Haemagglutinin content and type of vaccine
15 MF59 vs 11 non-adjuvanted:**

7.5 µg		15 µg		30 µg	
MF59	SA	MF59	SA	MF59	SA
(n=6)	(n=3)	(n=3)	(n=6)	(n=6)	(n=2)

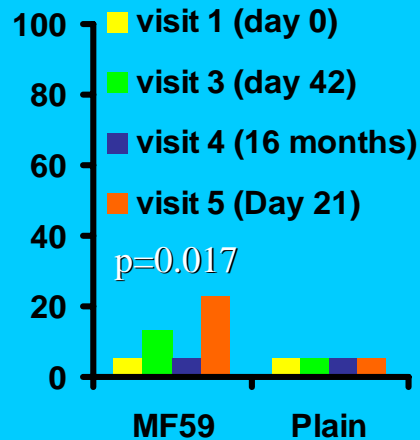
Adverse events	MF59	SA	p
Erythema ≥ 10 mm	9/15	0/11	0.004
Induration ≥ 10 mm	7/15	0/11	0.021
Pain			ns
Systemic features			ns

Baseline numbers with HI titres $\geq 1/40$, MN $\geq 1/20$, SRH $> 25\text{mm}^2$ and seroconversions

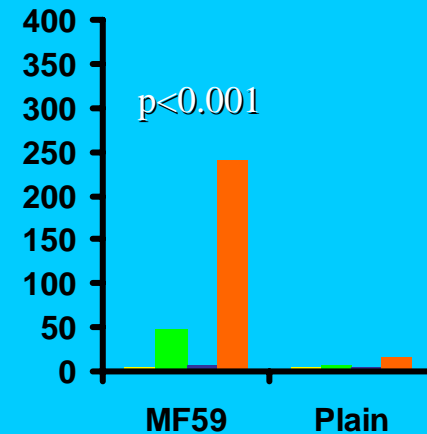
Assay	Day	7.5 μg		15 μg		30 μg		All		p Vaccine type
		MF59 (n=6)	SA (n=3)	MF59 (n=3)	SA (n=6)	MF59 (n=6)	SA (n=2)	MF59 (n=15)	SA (n=11)	
HI	0	0	0	0	0	0	0	0	0	1.0
	21	4	0	1	0	4	0	9	0	<0.001
MN	0	0	0	1	0	0	0	1	0	1.0
	21	6	0	3	3	6	2	15	5	<0.001
SRH H5N3	0	3	0	1	1	6	0	10	1	<0.001
	21	6	3	3	5	6	2	15	10	0.3
H5N1	0	1	0	1	0	0	0	2	0	0.11
	21	6	1	3	3	6	2	15	6	<0.001

GMTs of antibody at 16 months and response to revaccination

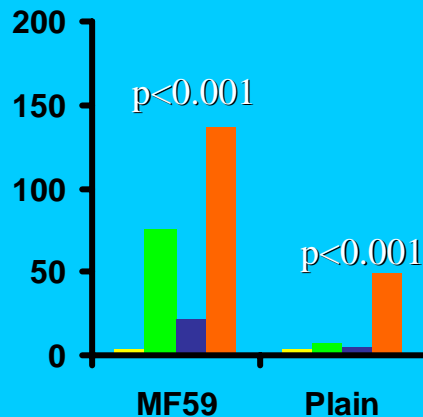
Haemagglutination inhibition (H5N3)



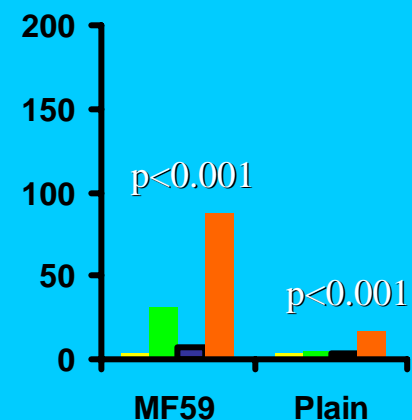
Microneutralisation (H5N3)



SRH (H5N3)



SRH (H5N1)



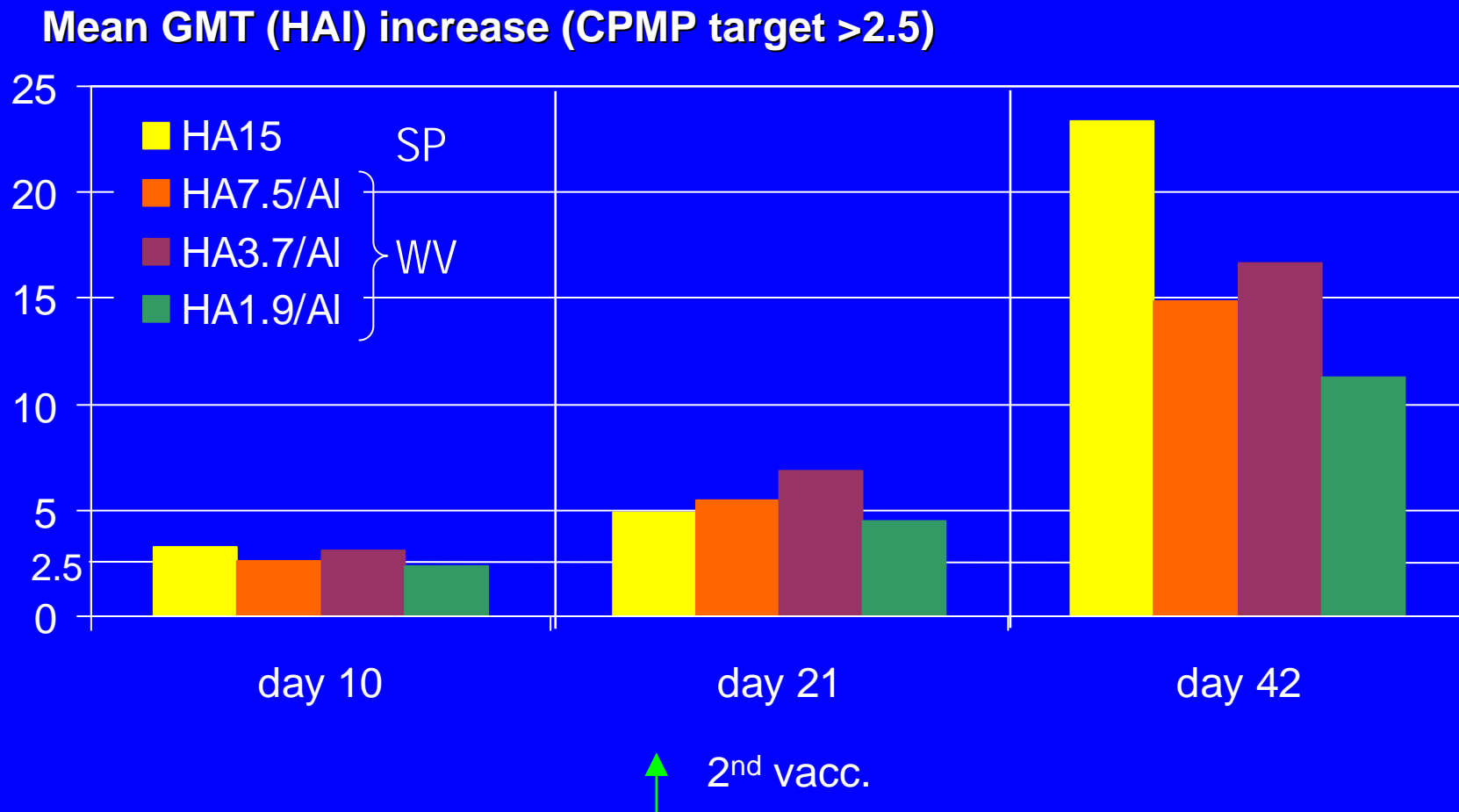
SRH results using A/Hong Kong/489/97 (H5N1) in relation to CPMP criteria

Haemagglutinin content and type of vaccine

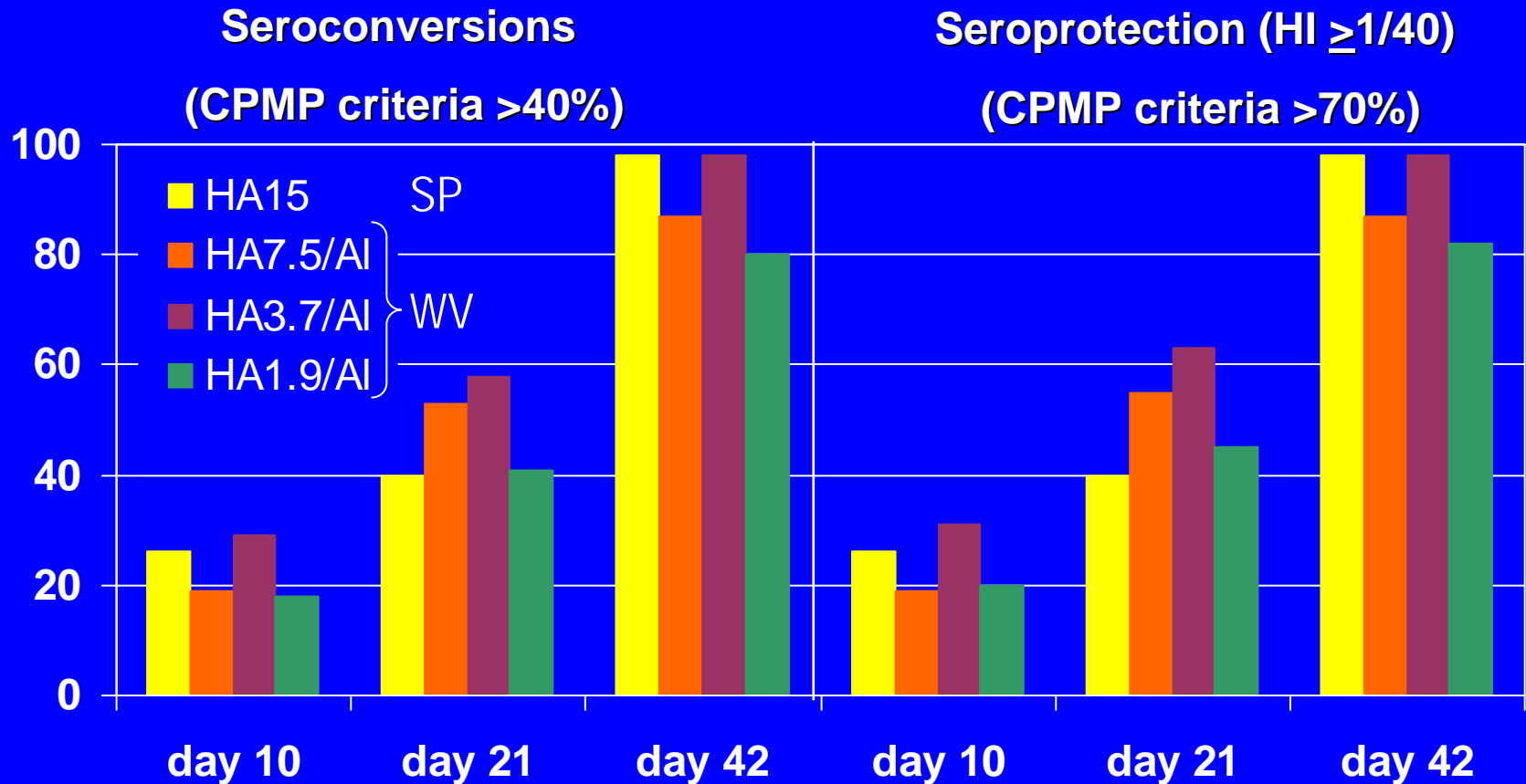
	7.5 µg		15 µg		30 µg	
	MF59	SA	MF59	SA	MF59	SA
	(n=6)	(n=3)	(n=3)	(n=6)	(n=6)	(n=2)
GMT						
Day 0 (Month 16)	9.6	4	8	4	5	4
Day 21	88	17	84	13	89	35
Mean GMT increase (>2.5)	9.1 †	4.3 †	10.5 †	3.3 †	17.8 †	8.8 †
% SRH titre >25mm² (>70%)	100 †	33	100 †	50	100 †	100 †
% seroconversions (>40%)	100 †	33	100 †	55 †	100 †	100 †

21 days after revaccination, GMTs in MF59 group were significantly higher in all tests

Clinical trial of A/Singapore/1/57 (H2N2) SP & WV vaccines: HAI GMT increases

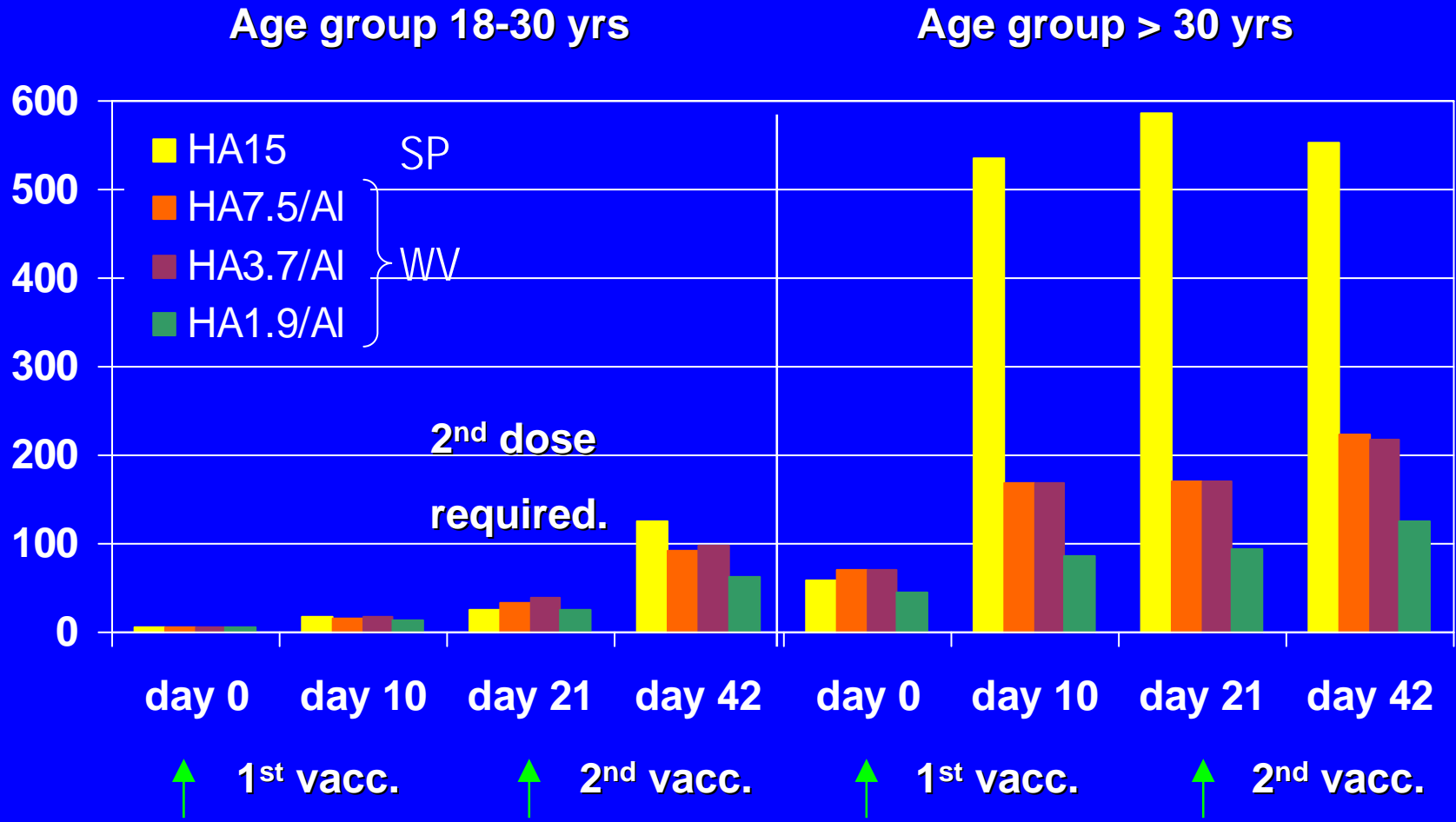


Trial of A/Singapore/57 (H2N2) vaccine: Seroconversion and seroprotection rates



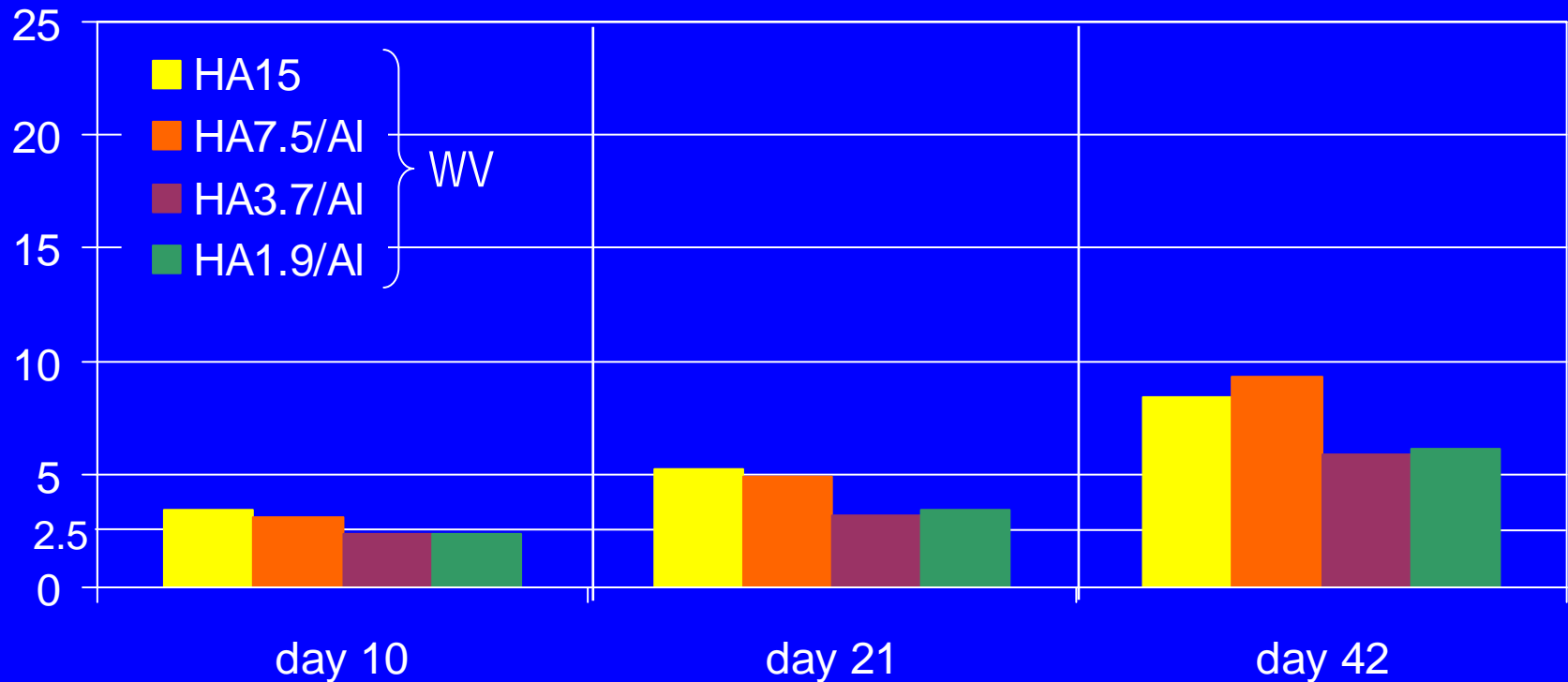
Clinical trial of H2N2 vaccine

Geometric mean HI titre according to age



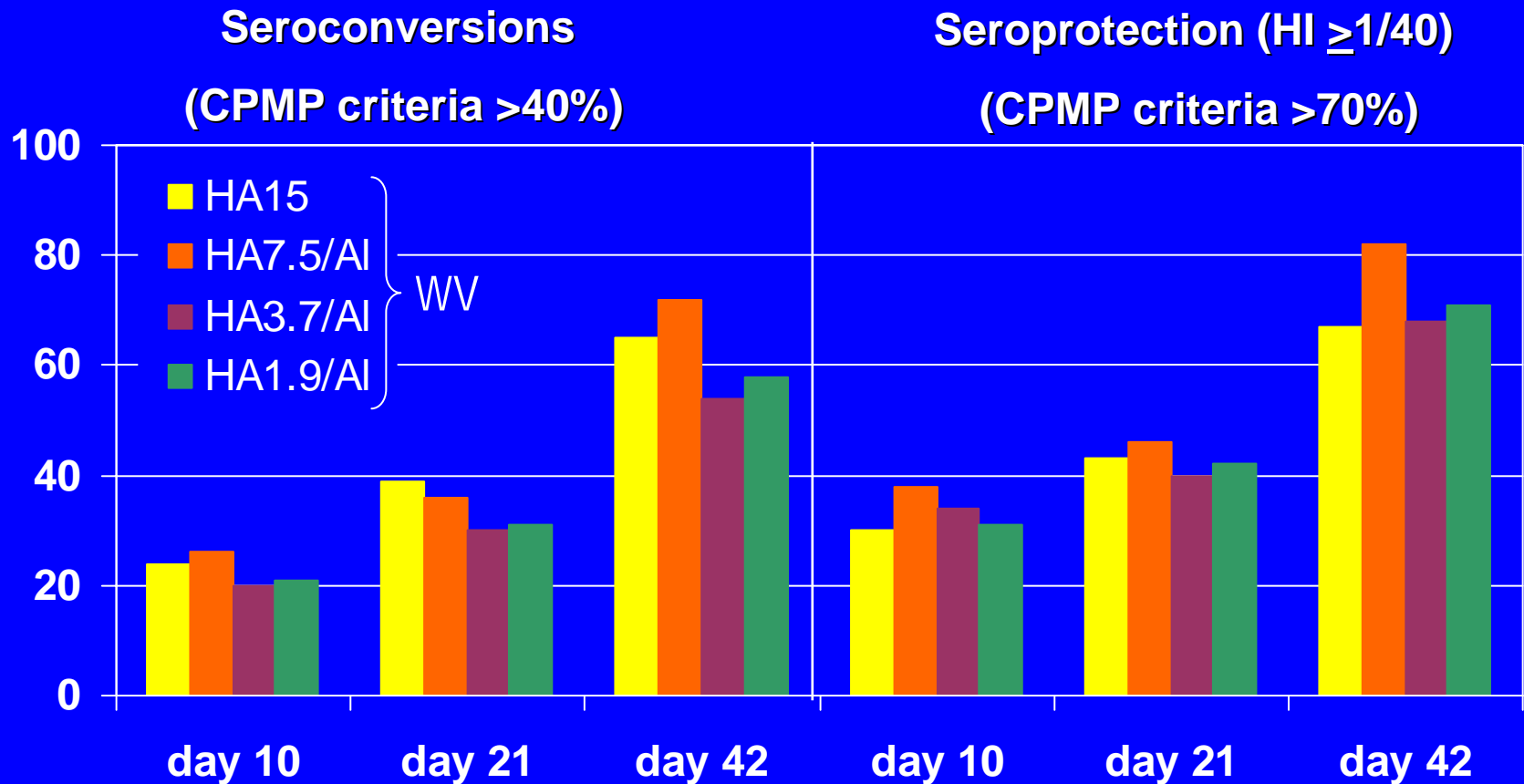
Clinical trial of A/Hong Kong/1073/99 (H9N2) WV vaccine: GMT increases

Mean GMT HAI increase (CPMP target >2.5)



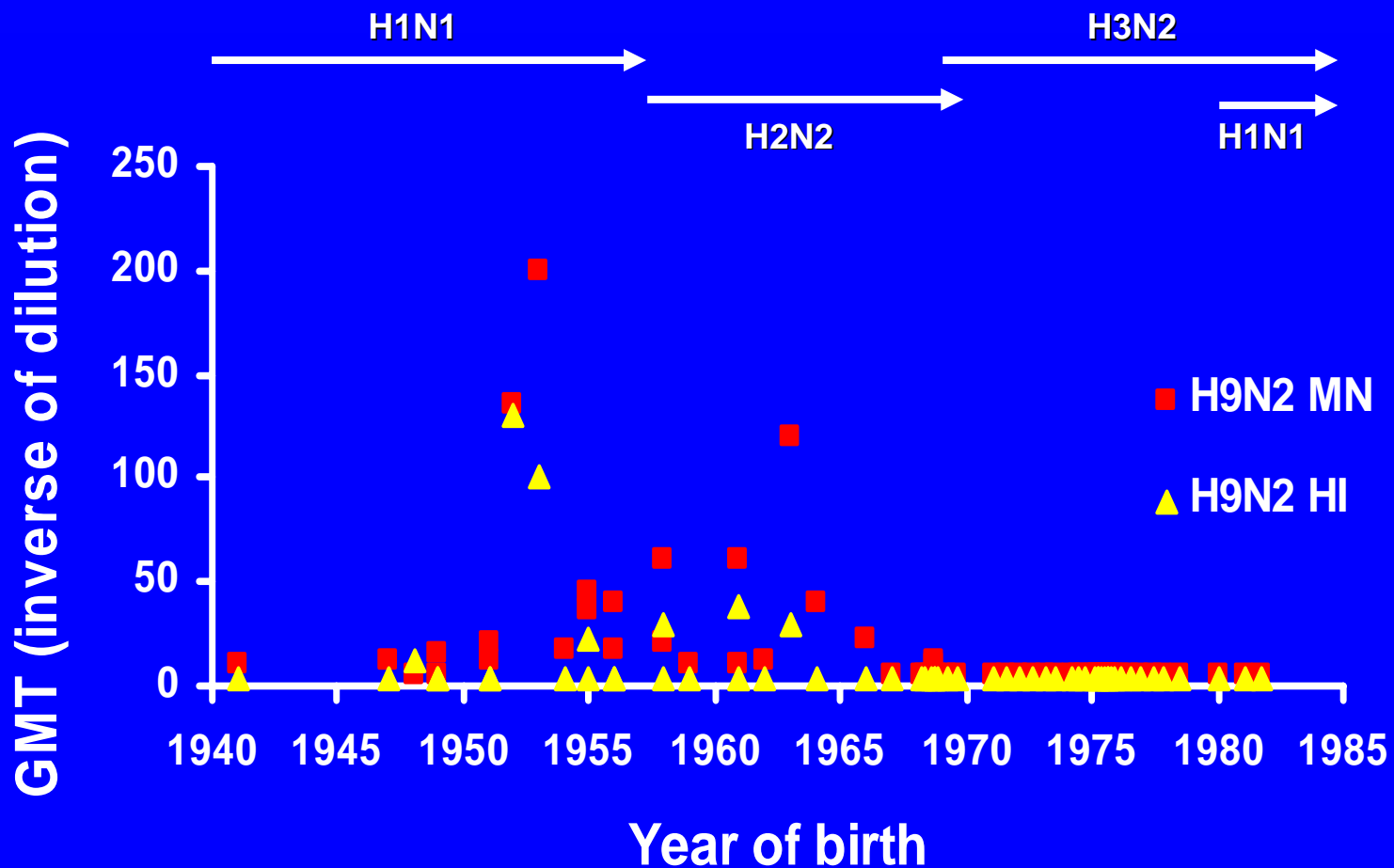
↑ 2nd vacc.

Trial of A/Hong Kong/1073/99 (H9N2) WV: Sero-conversion & -protection rates



Scatterplot of baseline H9N2 MN & HI titres against year of birth

Age-related detectable baseline antibody to A/Hong Kong/1073/99 (H9N2) by MN and HI



24/60 unexpectedly had age-related baseline antibody

Virus subtypes used in serological analysis of prevaccination sera

Virus	Subtype	Reason for use
A/Hong Kong/1073/99	H9N2	G1-like H9: vaccine strain. Responsible for human infection in Hong Kong.
H9N1 NIB-44 reassortant	H9N1	To investigate anti-H9 responses without potential N2 interference. G1-like H9 derived strain that is antigenically closely related to vaccine H9 component.
H7N2 X-15 reassortant ¹	H7N2	To investigate N2 responses. The N2 in H7N2 strain is derived from an N2 antigenically closely related to the vaccine N2 component
A/Sydney/5/97	H3N2	To investigate any H3 cross reaction (N2 antigen antigenically drifted from N2 vaccine antigen)
A/HK/1/ 68-like reassortant	H3N7	To investigate any earlier H3 cross reaction without potential N2 interference
A/Japan/57-like reassortant	H2N1	To investigate if any H2 cross-reaction. H2 viruses circulated widely in Europe from 1957- 68 when replaced by H3N2.
A/Beijing/262/95	H1N1	To investigate any H1 cross reaction

Clinical trial of H9N2 vaccine

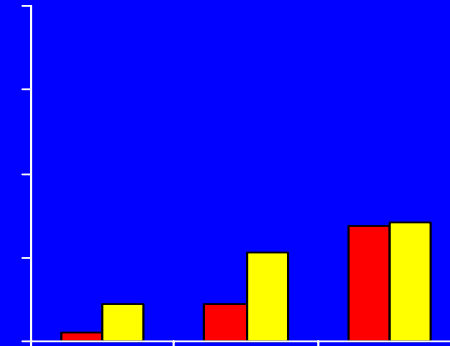
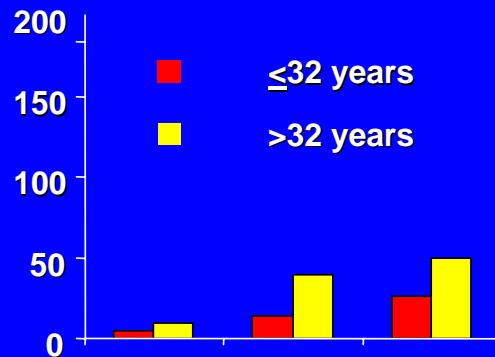
GMTs according to age

GMT (inverse of dilution)

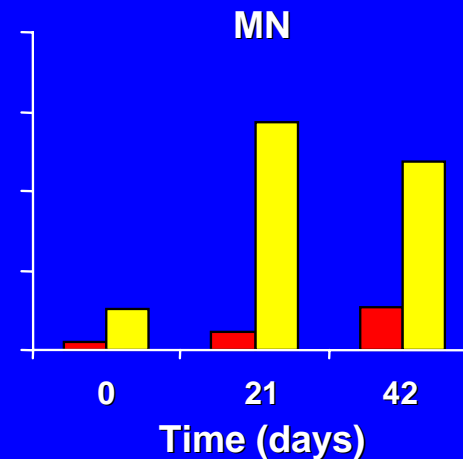
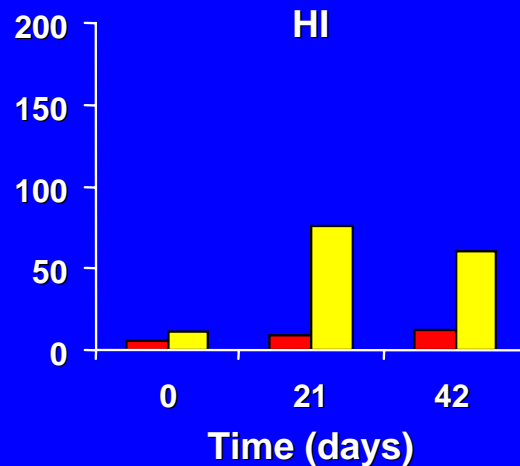
HI

MN

WV
vaccine



SA
vaccine



Comparison of GMTs in individuals aged ≤32 years and >32 years

HI : Day 21, p=0.0001 Day 42, p=0.002 MN: Day 21, p<0.0001 Day 42, p=0.006

HI results for A/Hong Kong/1073/99 (H9N2) in relation to CPMP criteria

	≤32 yrs of age		>32 yrs of age	
	WV (n=14)	SA (n=14)	WV (n=12)	SA (n=16)
GMT increase (>2.5)				
21 days	2.3	1.7	2.2	5.4†
42 days	6.9†	2.8†	3.0†	4.7†
Post-vaccination titre >1:40 (70%)				
0	0	0	2	4 (25%)
21 days	3 (21%)	2 (14%)	6 (50%)	12 (75%)†
42 days	6 (43%)	2 (14%)	8 (66%)	12 (75%)†
Seroconversions (>40%)				
21 days	5 (36%)	2 (14%)	6 (50%)†	9 (56%)†
42 days	9 (64%)†	5 (36%)	9 (75%)†	9 (56%)†

Local and systemic reactions to both H9N2 injections

	Haemagglutinin content and type of vaccine						p
	7.5 µg		15 µg		30 µg		
	WV (n=10)	SA (n=10)	WV (n=9)	SA (n=10)	WV (n=7)	SA (n=10)	
Local							
Pain							
None	7	6	4	10	4	10	0.01
Mild	3	3	5	0	3	0	0.012
Moderate	0	1	0	0	0	0	>0.999
Severe	0	0	0	0	0	0	..
Fever (≥38°C)	1	0	0	0	0	0	>0.999
Erythema	0	0	1	0	0	1	0.61
Induration	0	0	1	0	0	0	0.286
Systemic							
Chills	2	1	1	1	0	0	0.845
Myalgia	3	2	2	3	0	0	0.294
Headache	6	1	4	1	2	4	0.113
Nausea	4	1	0	0	0	0	0.015
Arthralgia	4	0	0	2	0	0	0.010
Diarrhoea	0	1	0	0	0	1	>0.999
Analgesia/antipyretic use	3	1	0	0	1	3	0.187

Summary – points to consider for design of a clinical protocol

1. Adverse events

- rHA, H5 & H9 antigens are generally well tolerated
- Adjuvants & WV vaccine evidently increase risk of local and possibly systemic adverse effects
- **Comment**
Candidate vaccines will need to be assessed in young children to assess tolerability

Summary – points to consider for design of a clinical protocol

2. Number of doses

- In immunologically naïve subjects at least two doses of vaccine containing a novel avian antigen are evidently required
 - The recent data from Germany suggests that this may hold true for H2N2, but older primed subjects may require only one dose
 - There is an age-related baseline cross-reacting antibody between H2 and H9, and a subsequent better response to H9 vaccine in older individuals
- **Comment**
 - *Two doses should be assessed by clinical trial*
 - *Effect of age on antibody responsiveness should always be considered*

Summary – points to consider for design of a clinical protocol

3. Dose range

- rHA – high dose range will need to be explored on basis of limited data
 - Limited data with novel antigens suggest a relatively flat dose-response
 - Dose response may be affected by the relative quantities of adjuvant
- Comment
 - *With conventionally prepared material, there seems little point in using 'high' doses of HA, since supplies will be limited any way*
 - *Range needs to be explored with and without adjuvants to ensure that adjuvants really augment the immune response*

Summary – points to consider for design of a clinical protocol

4. Dose interval

- **Recent studies with rHA suggests that prolonging the interval is not beneficial and is probably detrimental**
- **Comment**
 - *'Accelerated' 2 dose regimens should be examined*
 - *Interval between doses shouldn't exceed 21 days – a short interval between doses is more likely to achieve protection sooner*

Summary – points to consider for design of a clinical protocol

5. Antigenicity

- rHA appears particularly poor in immunologically naïve
 - Frequency of response to rHA depends on total amount of antigen delivered – not obviously the case on basis of limited data with conventional and adjuvanted HA
 - H5 appears to be particularly poor as an antigen –
?true or due to the limited amount of data available
- Comment
 - H5 still poses a pandemic threat – further work with H5 is required

Summary – points to consider for design of a clinical protocol

6. Formulations/adjuvants

- Few adjuvants have been examined and none head-to-head
 - Whole virion vaccine not been compared with MF59 adjuvanted material
 - Data on AlPO_4 and small quantities of WVV and Split vaccine looks promising
 - ?Paradoxical effect with MF59 – larger quantities of antigen evoke lower titres
- Comment
 - Large multicentre studies incorporating materials from different vaccine manufacturers are urgently required
 - Need to explore the relationship of MF59 with avian antigens in more detail

Summary – points to consider for design of a clinical protocol

7. Surrogate vaccines

- May closely resemble wild virus in laboratory tests but may evoke significantly lower titres to wild-type virus in comparison to the vaccine strain
- Comment
 - Attenuated high growth containing the 'wild-type HA' should be used whenever possible

Summary – points to consider for design of a clinical protocol

8. Antibody tests/CPMP criteria/harmonisation

- Are CPMP criteria stringent enough? - a relatively poor antigen can pass
- Tests to assess the response to novel antigens ? vary between centres
- H5 HI test insensitive
- No NA antibody data

• Comment

- Are new criteria for pandemic vaccines warranted?
- International collaboration required – as with SARS
- Several antibody tests should be incorporated, including NA testing: need also to consider use of alternative erythrocytes

Summary – points to consider for design of a clinical protocol

8. Antibody persistence

- Only one study – with very small numbers of subjects - has considered antibody persistence and revaccination
- Comment
 - Role of boosters needs to be considered in more detail

9. Age effect

- Data from H9 study indicate that there may be an age-effect due to cross-reacting antibodies
- Comment
 - Age effect needs to be explored in future trials
 - Children and the elderly need to be included in future studies

Summary – points to consider for design of a clinical protocol

10. Statistics

- **With statistical input useful information has been obtained from relatively small studies concerning the role of dose, vaccine type, age, etc**
- **Possible adverse effects from novel adjuvants should be taken into consideration when designing studies**
- **Comment**
 - **There needs to be consensus about the most appropriate size of Phase I and II studies**