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 (µg)
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 ± MF59)	65 (18-40)	6	2 (d0, 21)	 (7.5, 7.5) (15, 15) (30, 30)
Stephenson 2003	H5N3 (SA ± 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 ≥1:80
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 (µg)
Hehme 2001	H2N2 (WV + $AlPO_4$)* H2N2 (SP)	196 (18-30)	4	2 (d0, 21)	(1.9, 3.8, 7.5) (15)
	H9N2 (WV + $AlPO_4$)* 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)

*$AlPO_4$: 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 20µg doses to 56 subjects .
    - 2/28 receiving 10 µg dose developed VN Ab ≥1:80.
    - 6/28 receiving 20 µg dose developed VN Ab ≥1:80.

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

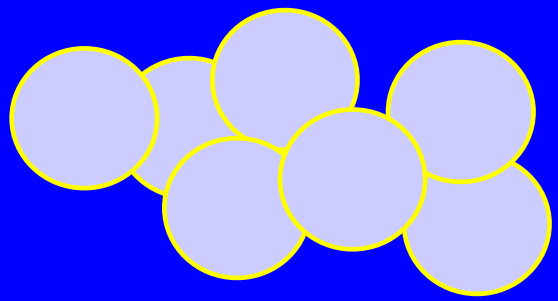
Dose 1/ Dose 2 (µg)	No. with 156 MN response* /No. tested when given vaccine at intervals of: 21	28	42	Any (%)	≥4-fold 483 EIA response
25/25	1/10	2/10	2/9	5/29 (17)	5/24 (21)
45/45	1/10	4/9	3/10	8/29 (28)	7/24 (29
90/90	5/9	6/10	4/10	15/29 (52)	8/19 (42)
90/10	4/10	4/10	2/10	10/30 (33)	8/23 (35)
Any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

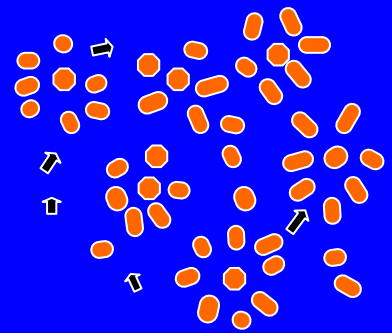
A/Duck/Singapore/97 (H5N3) MF59 adjuvanted vaccine

MF 59 oil in water emulsion



+

H5N3 surface antigen



Oil phase:	**9.75 mg Squalene (cholesterol metabolite)**	**+**	**1.175 mg sorbitan trioleate (oil soluble surfactant)**
Water phase:	**1.175mg Polysorbate 80 (Water soluble surfactant)**	**+**	**water in citrate buffer**

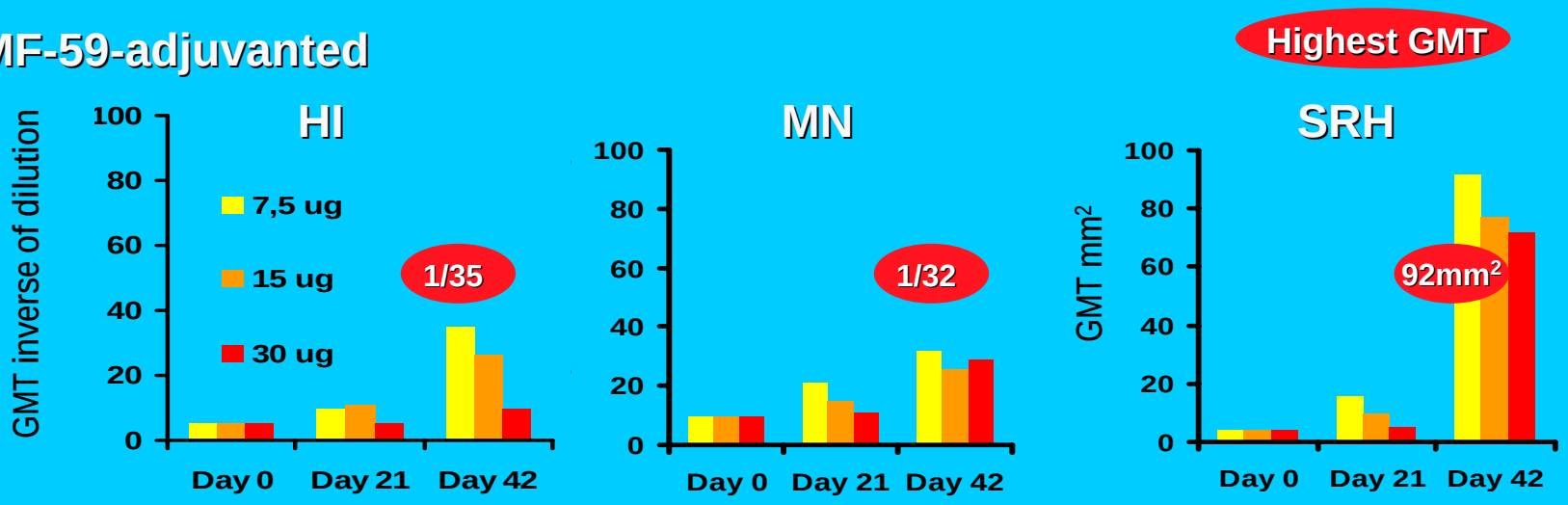
Local and systemic reactions to both injections

	Haemagglutinin content and type of vaccine						
	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)	p
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

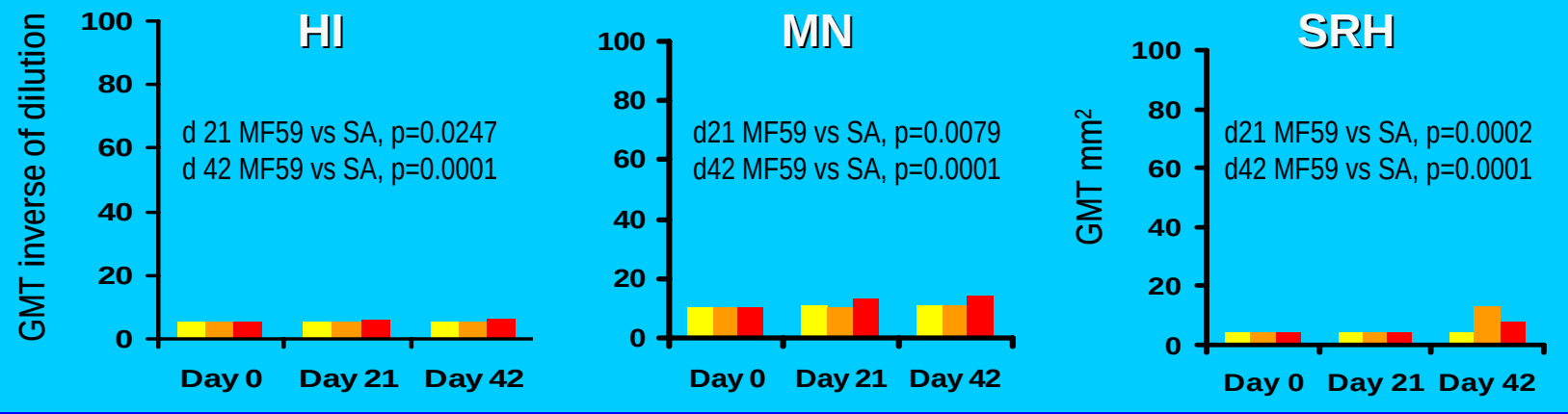
† Moderate & severe pain 9/32 (28%) vs 0/33

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

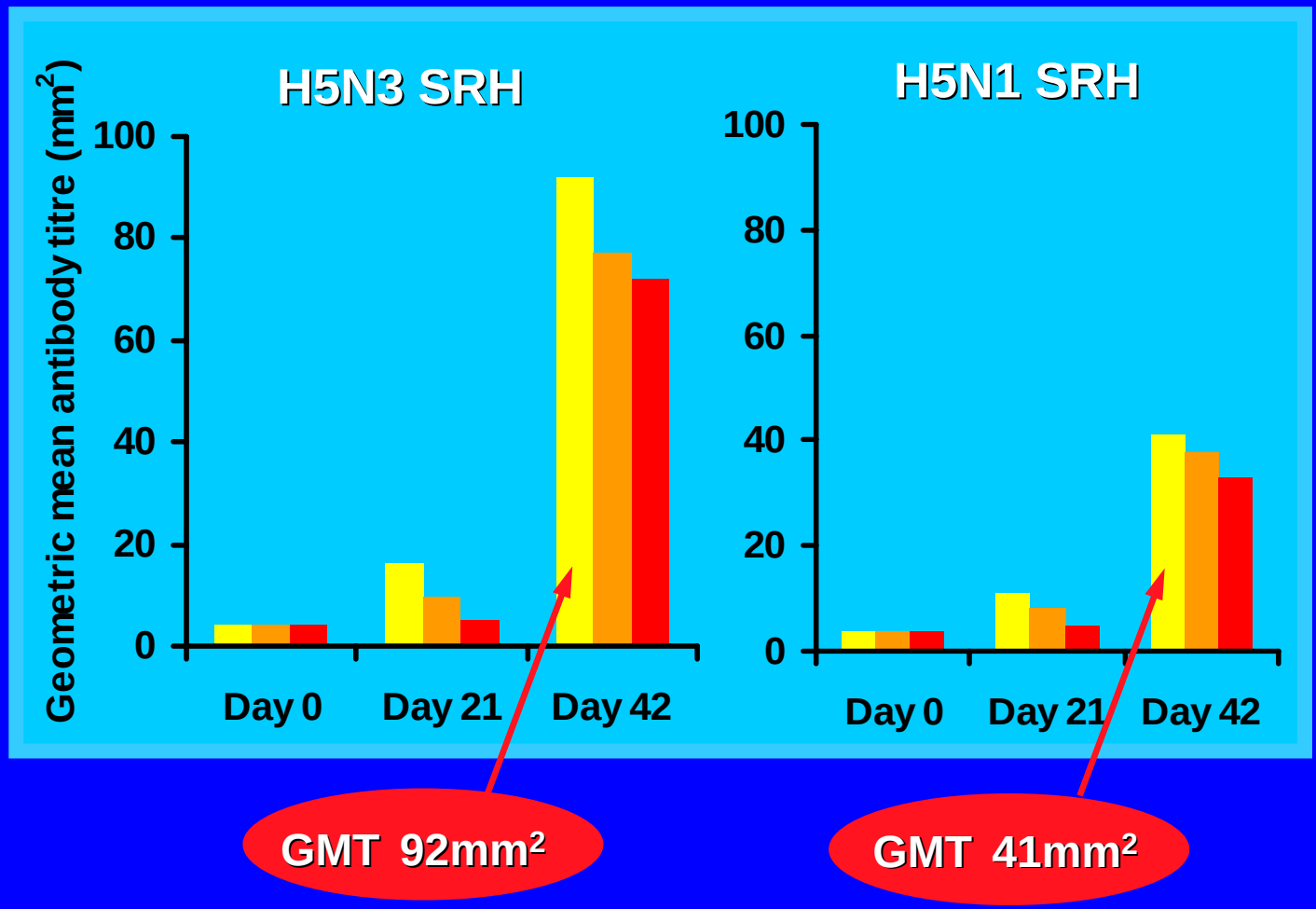
MF-59-adjuvanted



Non-adjuvanted



H5N3 & H5N1 SRH GMTs (mm^2) to MF-59 adjuvanted vaccine



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 (n=10)	SA (n=11)	MF59 (n=11)	SA (n=11)	MF59 (n=11)	SA (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**

Stephenson *et al* Vaccine 2003;21:1687-93.

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:

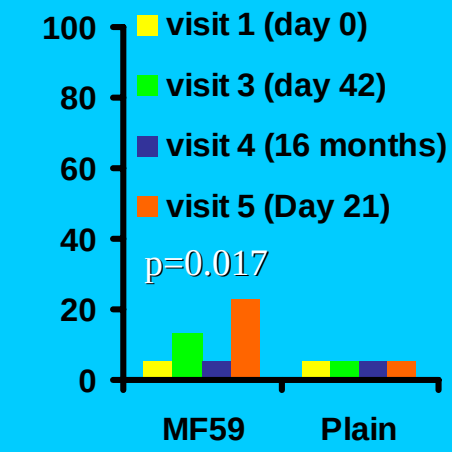
7.5 µg		15 µg		30 µg	
MF59 (n=6)	SA (n=3)	MF59 (n=3)	SA (n=6)	MF59 (n=6)	SA (n=2)

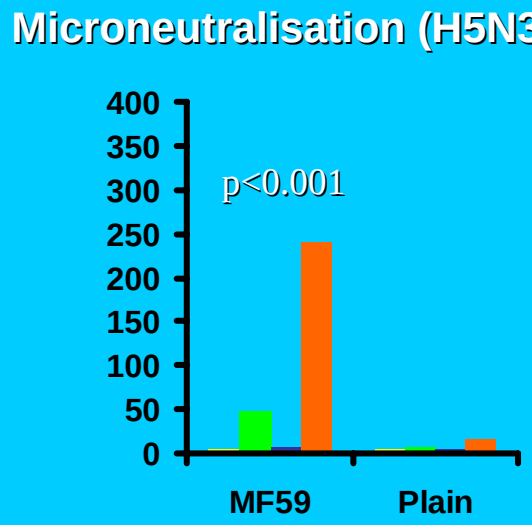
Adverse events	MF59	SA	p
Erythema ≥10mm	9/15	0/11	0.004
Induration ≥10mm	7/15	0/11	0.021
Pain			ns
Systemic features			ns

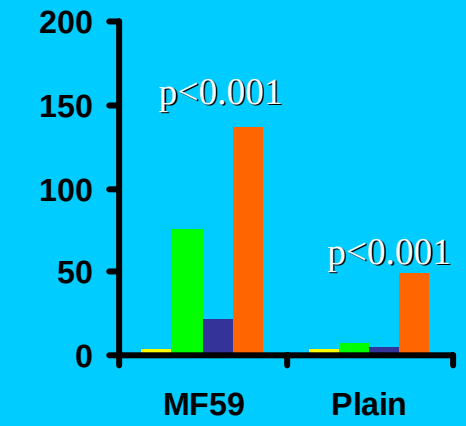
Baseline numbers with HI titres ≥1/40, MN≥1/20, SRH>25mm² and seroconversions

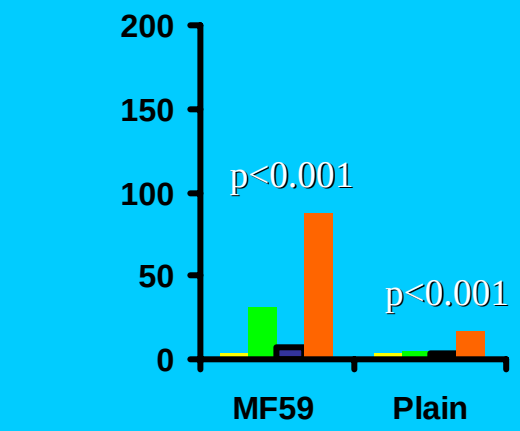
Assay	Day	7.5 µg MF59 (n=6)	7.5 µg SA (n=3)	15 µg MF59 (n=3)	15 µg SA (n=6)	30 µg MF59 (n=6)	30 µg SA (n=2)	All MF59 (n=15)	All SA (n=11)	p Vaccine type
HI	0	0	0	0	0	0	0	0	0	1.0
	21	4	0	1	0	4	0	9	0	<0.001
MN	0	0	0	1	0	0	0	1	0	1.0
	21	6	0	3	3	6	2	15	5	<0.001
SRH H5N3	0	3	0	1	1	6	0	10	1	<0.001
	21	6	3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







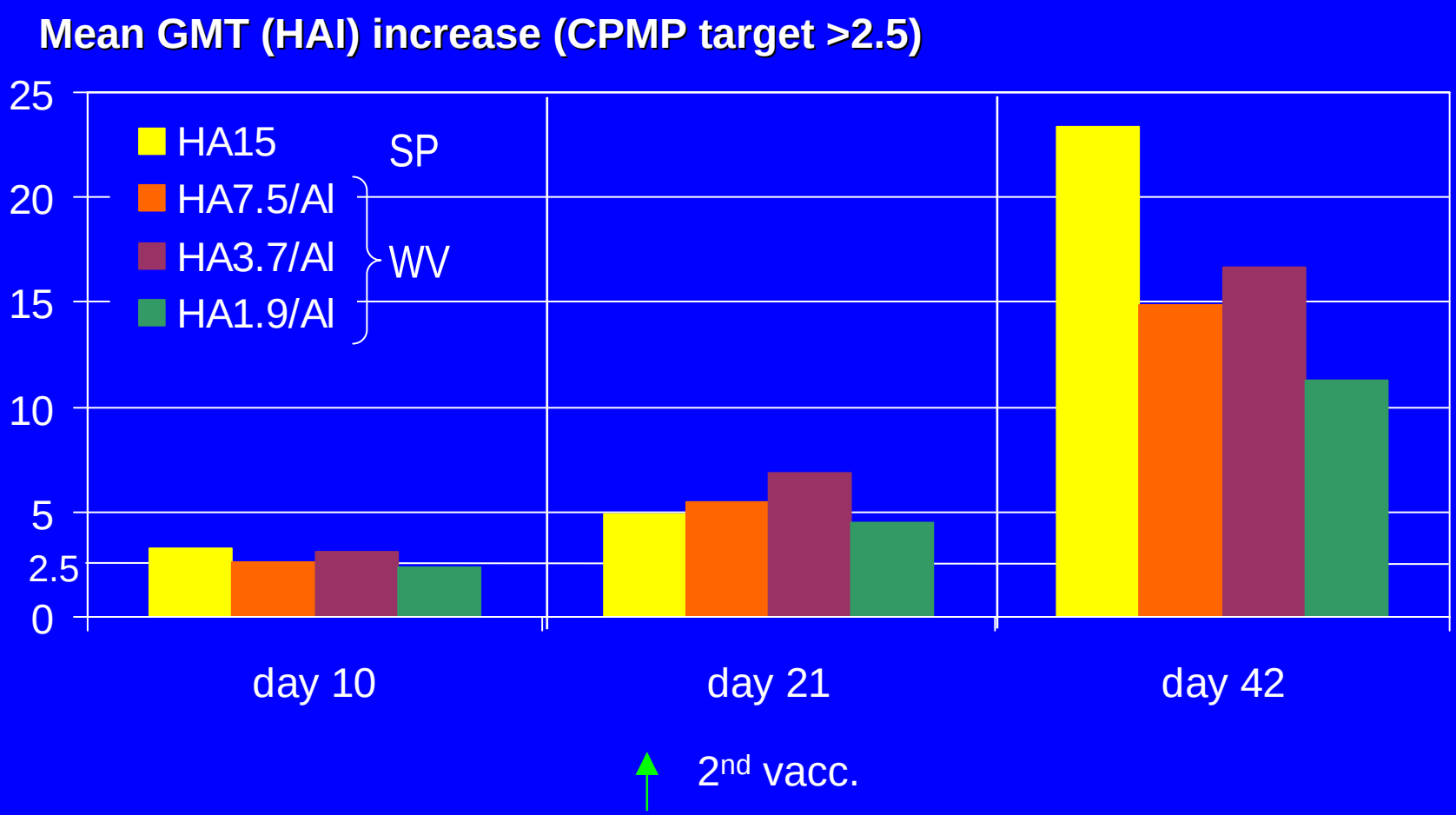


SRH results using A/Hong Kong/489/97 (H5N1) in relation to CPMP criteria

	Haemagglutinin content and type of vaccine					
	7.5 µg		15 µg		30 µg	
	MF59 (n=6)	SA (n=3)	MF59 (n=3)	SA (n=6)	MF59 (n=6)	SA (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 >25mm^2 (>70%)	100 †	33	100 †	50	100 †	100 †
% seroconversions (>40%)	100 †	33	100 †	55 †	100 †	100 †

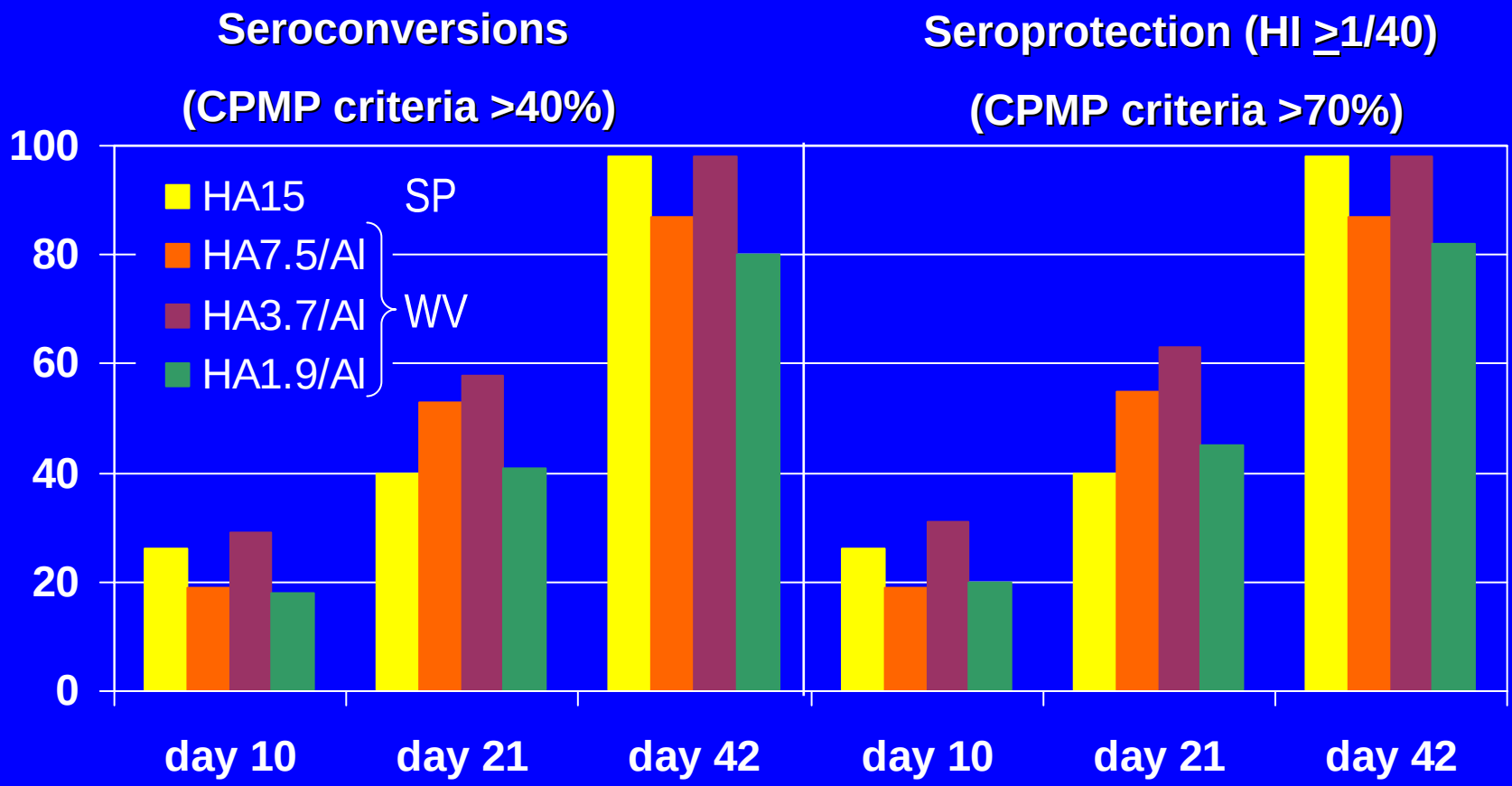
21 days after revaccination, GMTs in MF59 group were significantly higher in all tests

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



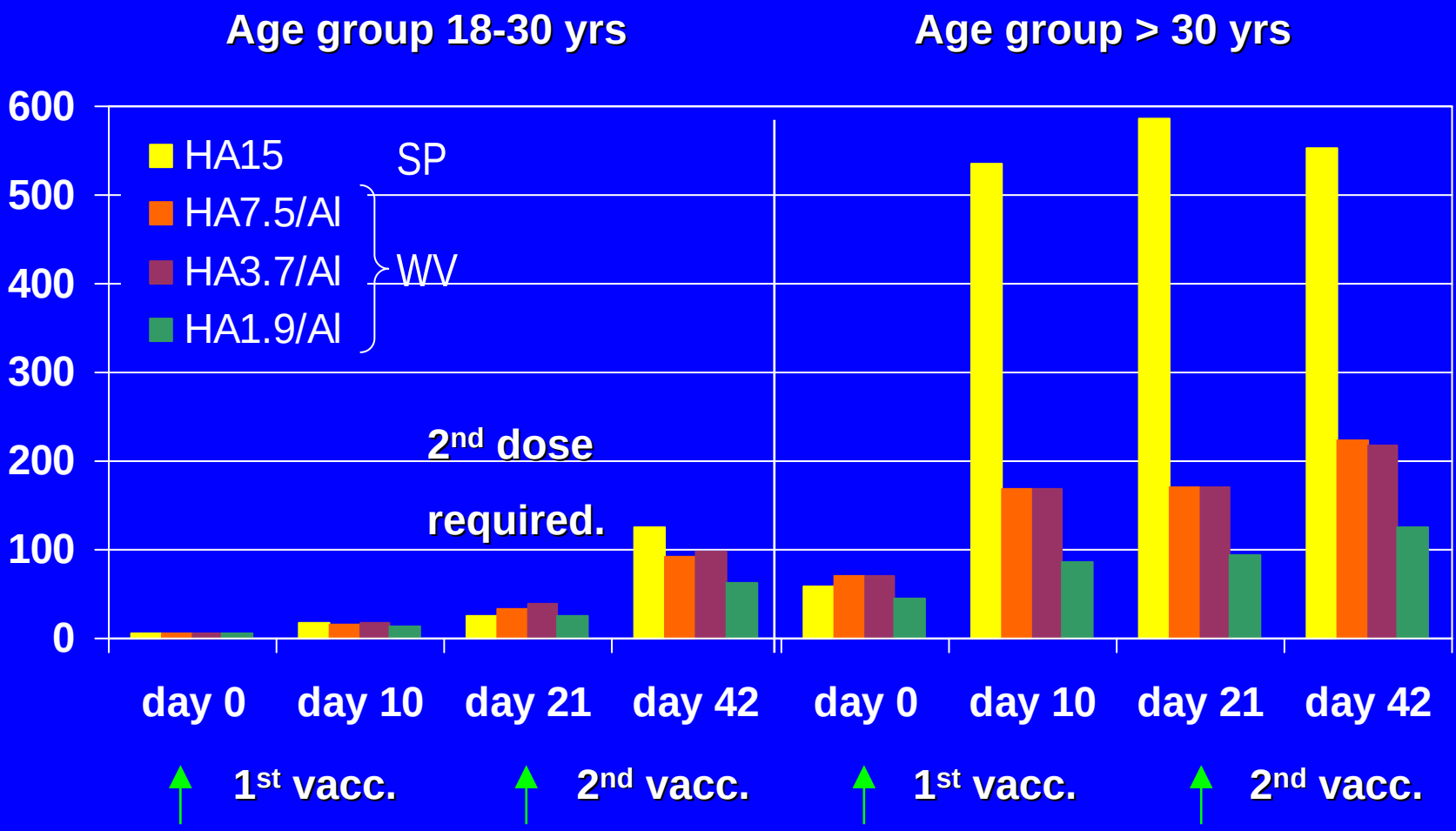
N Hehme et al, Med Microbiol Immunol 2002:191:203-8)

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



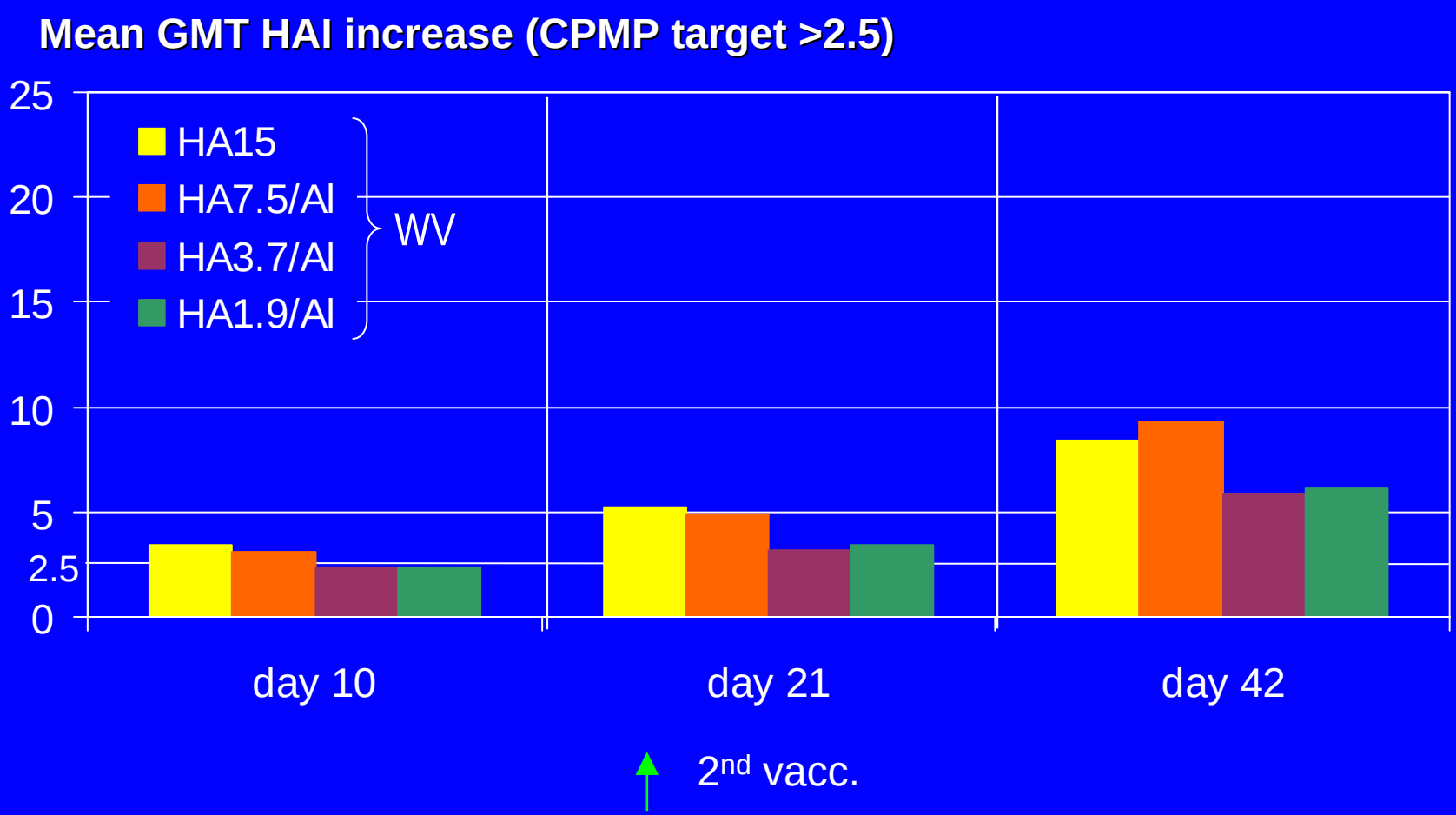
N Hehme et al, Med Microbiol Immunol 2002:191:203-8)

Clinical trial of H2N2 vaccine
Geometric mean HI titre according to age



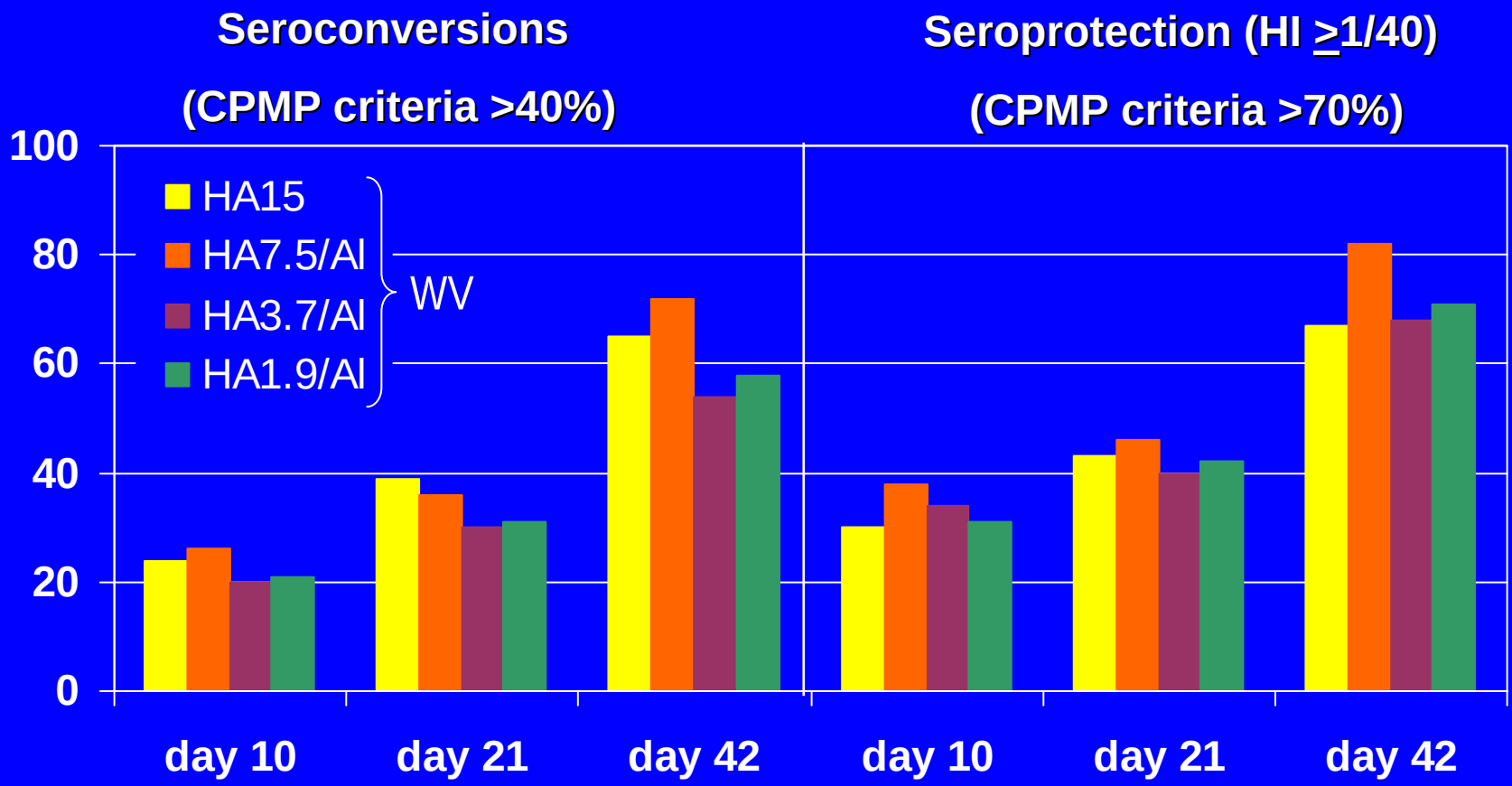
N Hehme et al, Med Microbiol Immunol 2002:191:203-8)

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



N Hehme et al, Med Microbiol Immunol 2002:191:203-8)

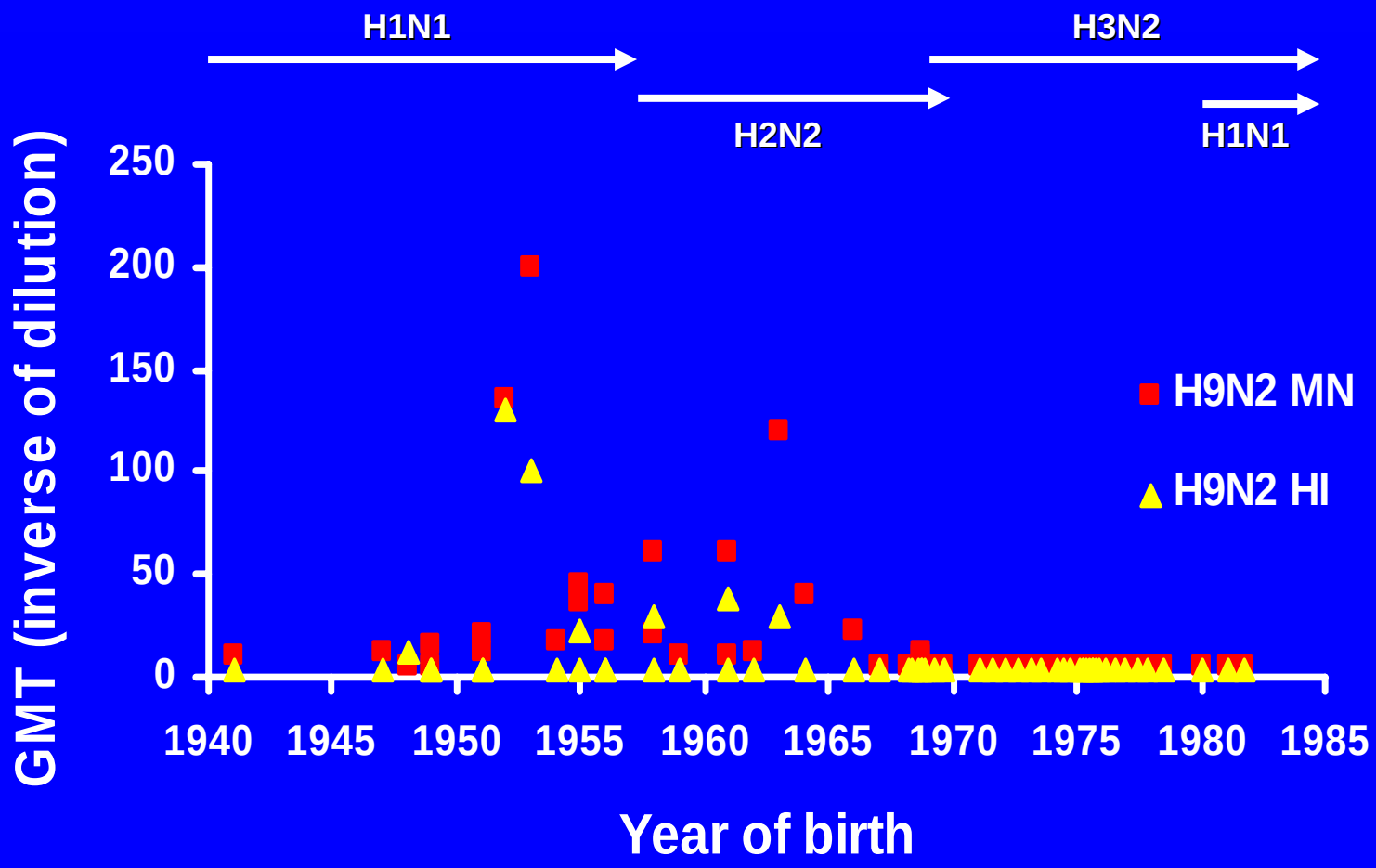
Trial of A/Hong Kong/1073/99 (H9N2) WVV: Sero-conversion & -protection rates



N Hehme et al, Med Microbiol Immunol 2002:191:203-8)

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



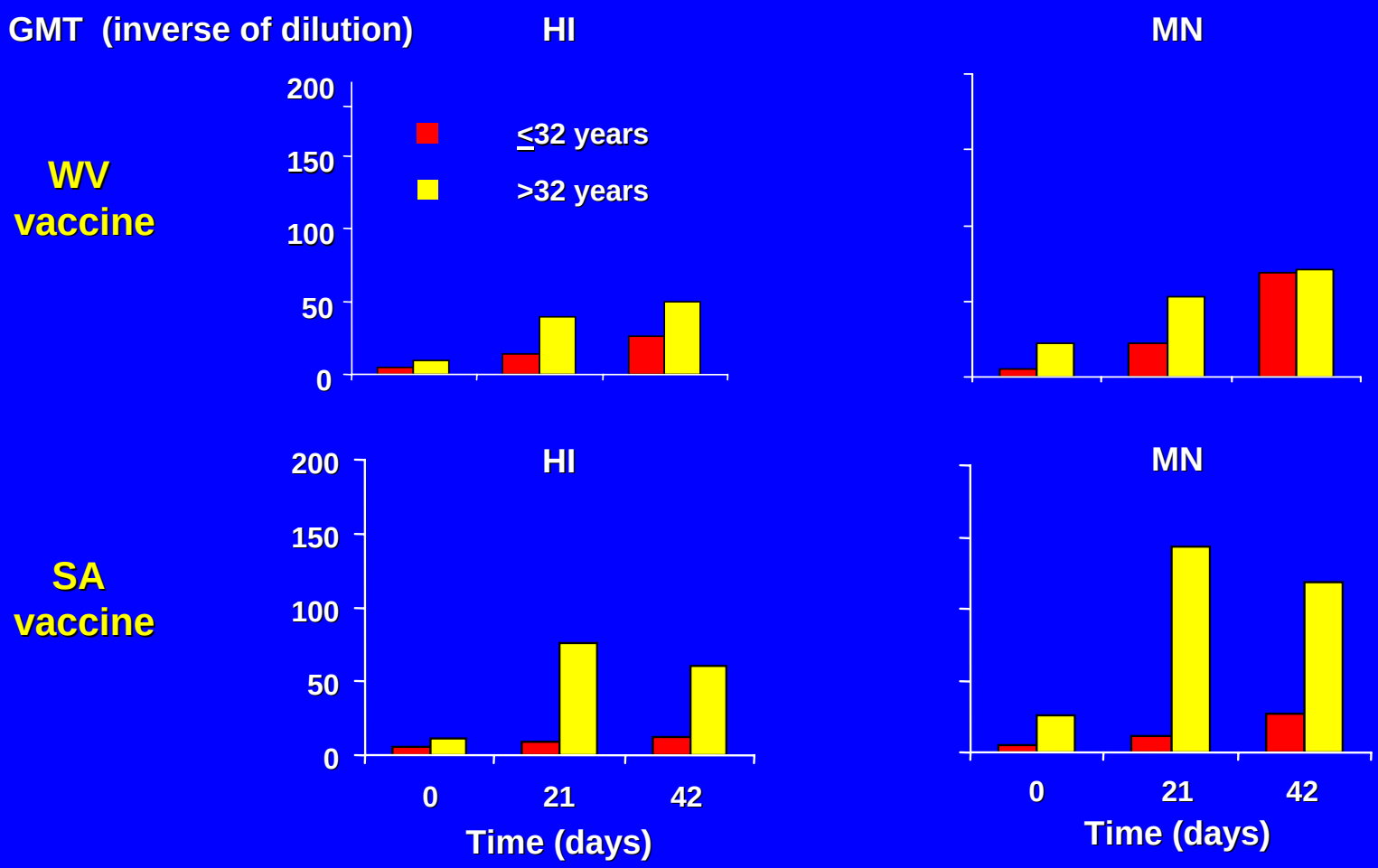
24/60 unexpectedly had age-related baseline antibody

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

Stephenson et al, Lancet 2003 In press.

Clinical trial of H9N2 vaccine GMTs according to age



Comparison of GMTs in 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						
	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)	p
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 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