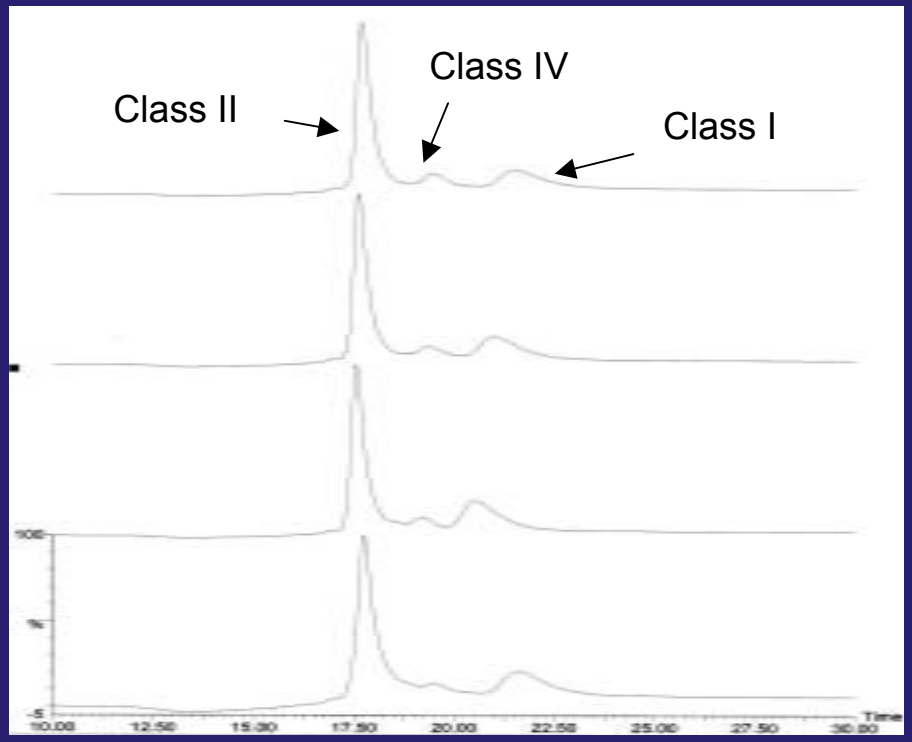
Proteosome™ Adjuvants for Nasal Vaccines Against Influenza and Plague

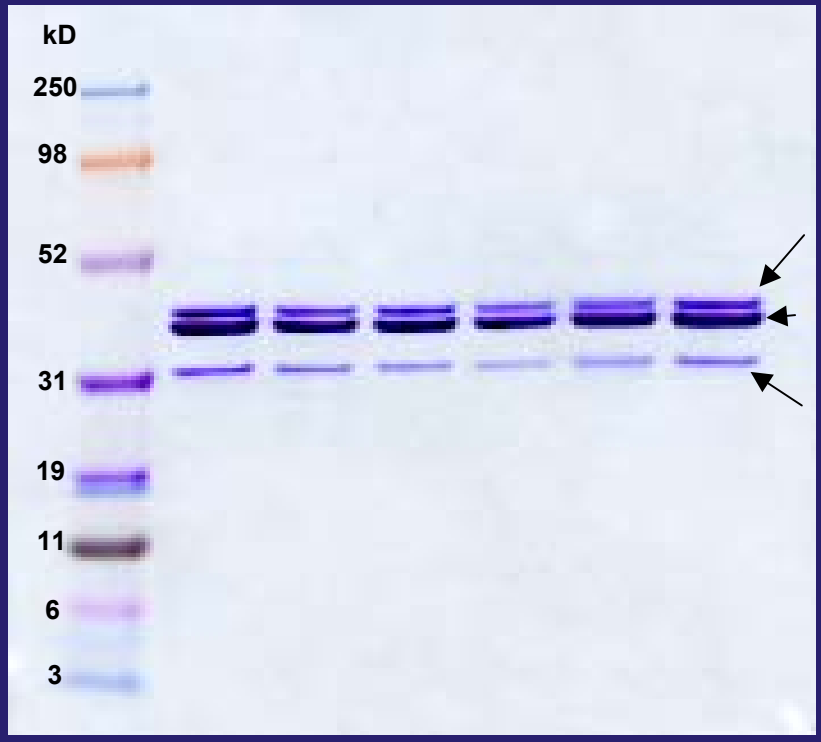
December 2003

Proteosome™ Technology Concept

- **Nanospheres of:**
  - Purified *N. mening.* outer membrane proteins classes 1, 2, and 4
  - ≥ 70% class 2 (PorB)
- **Immunostimulatory:**
  - PorB upregulates MHC class I & II, as well as B7.2 on APC via TLR 2
  - Member of class of "innate immunity" adjuvants acting via TLR (dsRNA, LPS, CpG, etc.)
  - Shifts T cell response to a Th1-type pattern
- **Effective mucosal delivery of protein,peptide, & polysaccharide antigens in animals and man.**

Proteosome™ System

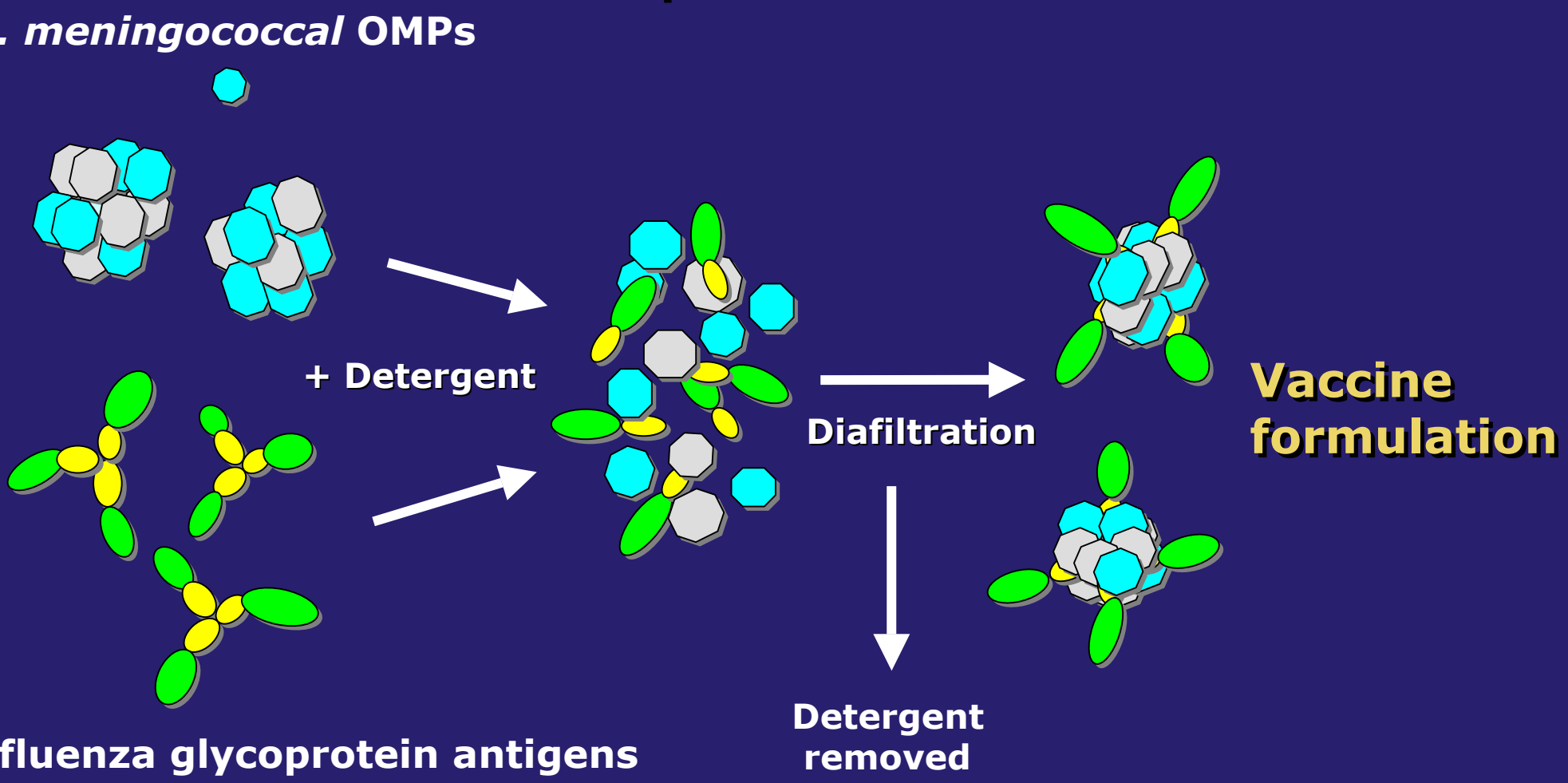




Proteosome™ Manufacture

- Fully-characterized master and working seeds
- Gentle process of sequential liquid phase extractions and fractionations.
- Readily scalable, now at 10 - 20% of market scale
- Consistency in > 25 lots (10 GMP) monitored by:
  - SDS/Page: Coomassie and Western
  - RP-HPLC
  - Mass spec on whole product and HPLC-isolated OMPs (m.w. of OMPs agrees with gene sequence prediction w/i 4 a.m.u.)
  - AAA and N-terminal sequencing on 8 lots
  - Residuals:
    - < 1.0% bacterial nucleic acids (actually < 0.1%)
    - Mean 1.3% LOS
- Sterile filterable, bulk intermediate storage at - 20º C.
- Stability by structural methods and retained adjuvant activity:
  - ≥ 3 yrs. at -20º
  - ≥ 1 month at 40ºC

FluINsure™ Vaccine
Preparation

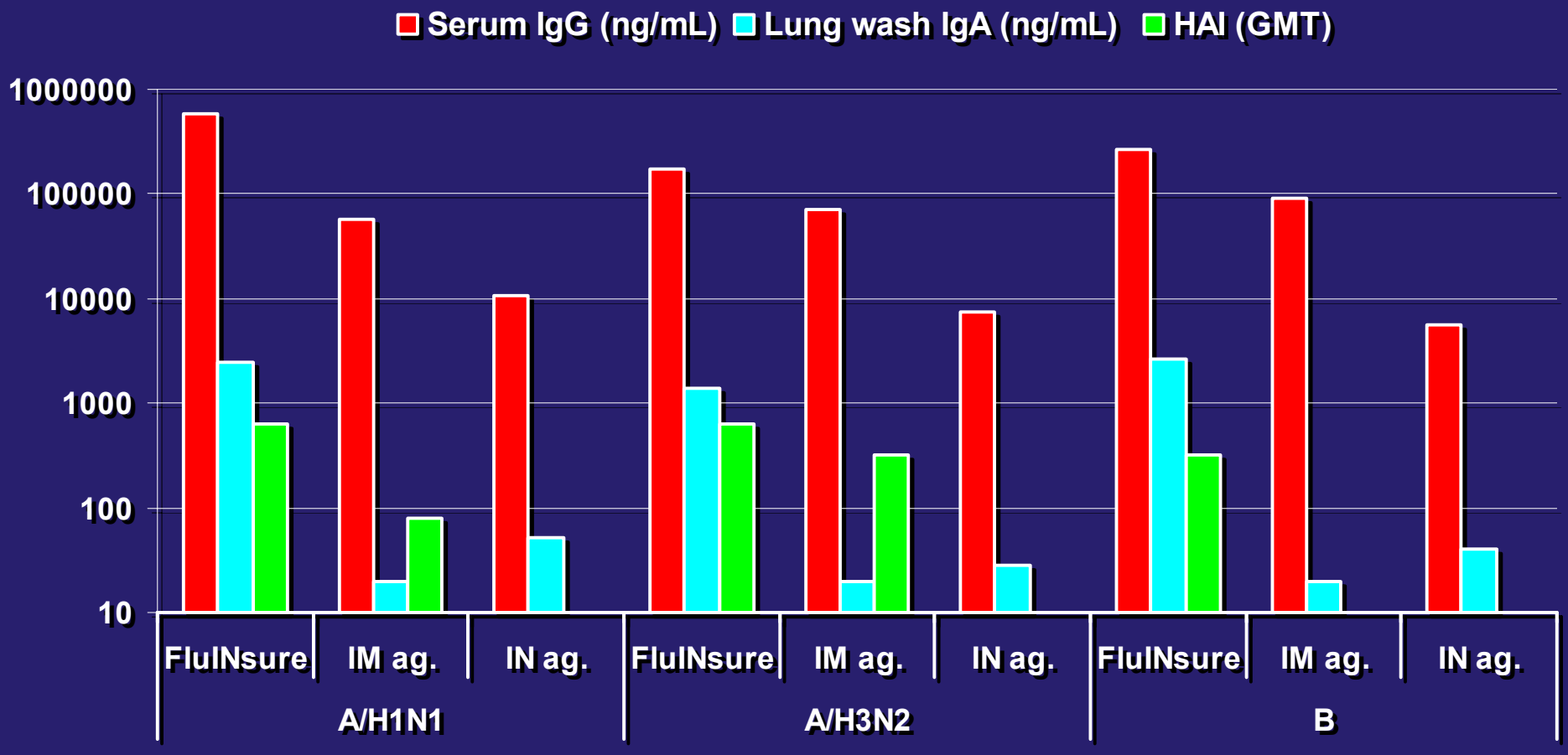


- Can incorporate formalin or beta-propiolactone inactivated egg-grown virus, tissue culture virus, or recombinant (e.g., baculovirus) antigens

FluINsure™ Vaccine

- **Stability:**
  - Retention of mouse and human immunogenicity, particle size, and HA content by quantitative SRID
  - 2 – 8º C for > 9 months
  - 25º C for > 1 month
- **Formulation:**
  - Initial lots thimerosal-preserved
  - Preservative-free, 0.22 µm filterable formulations now available for clinic

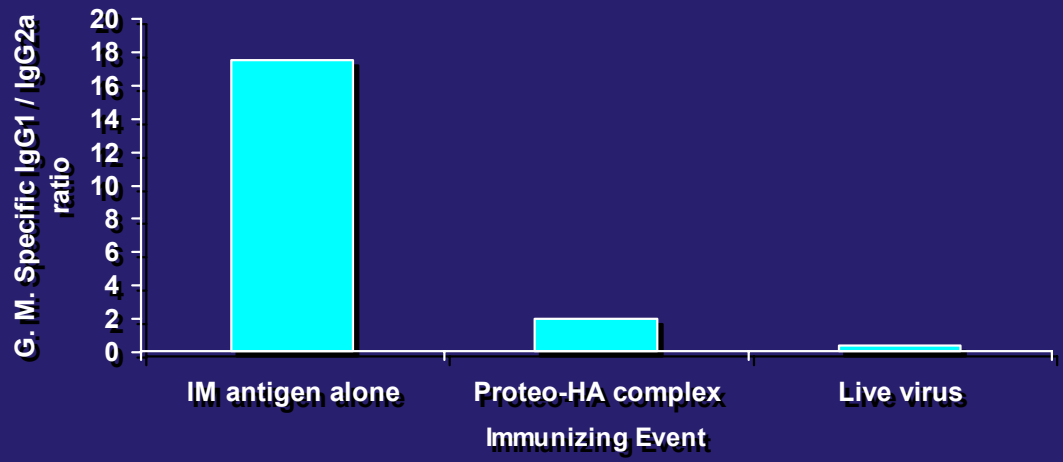
Intranasal FluINsure™
Typical Immunogenicity in Rodents



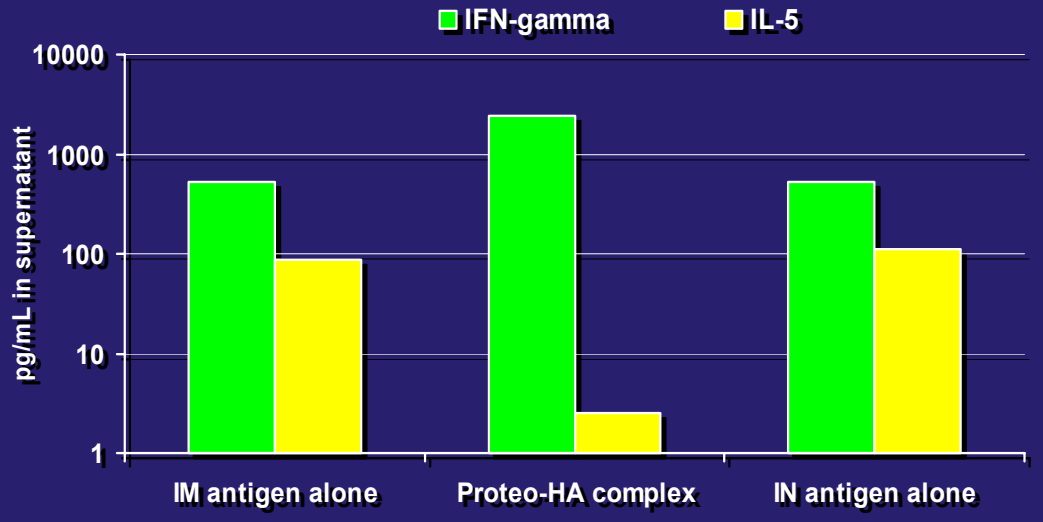
Geo. mean data, groups of 10 mice given two (2) IN or IM doses containing 1 µg of each HA at a 14 –day interval and sacrificed day 28.

Intranasal FluINsure™
Effects on the T cell Response in Mice

- IgG1 / IgG2a ratio in antibody response more closely mimics live virus infection after FluINsure than IM vaccine.

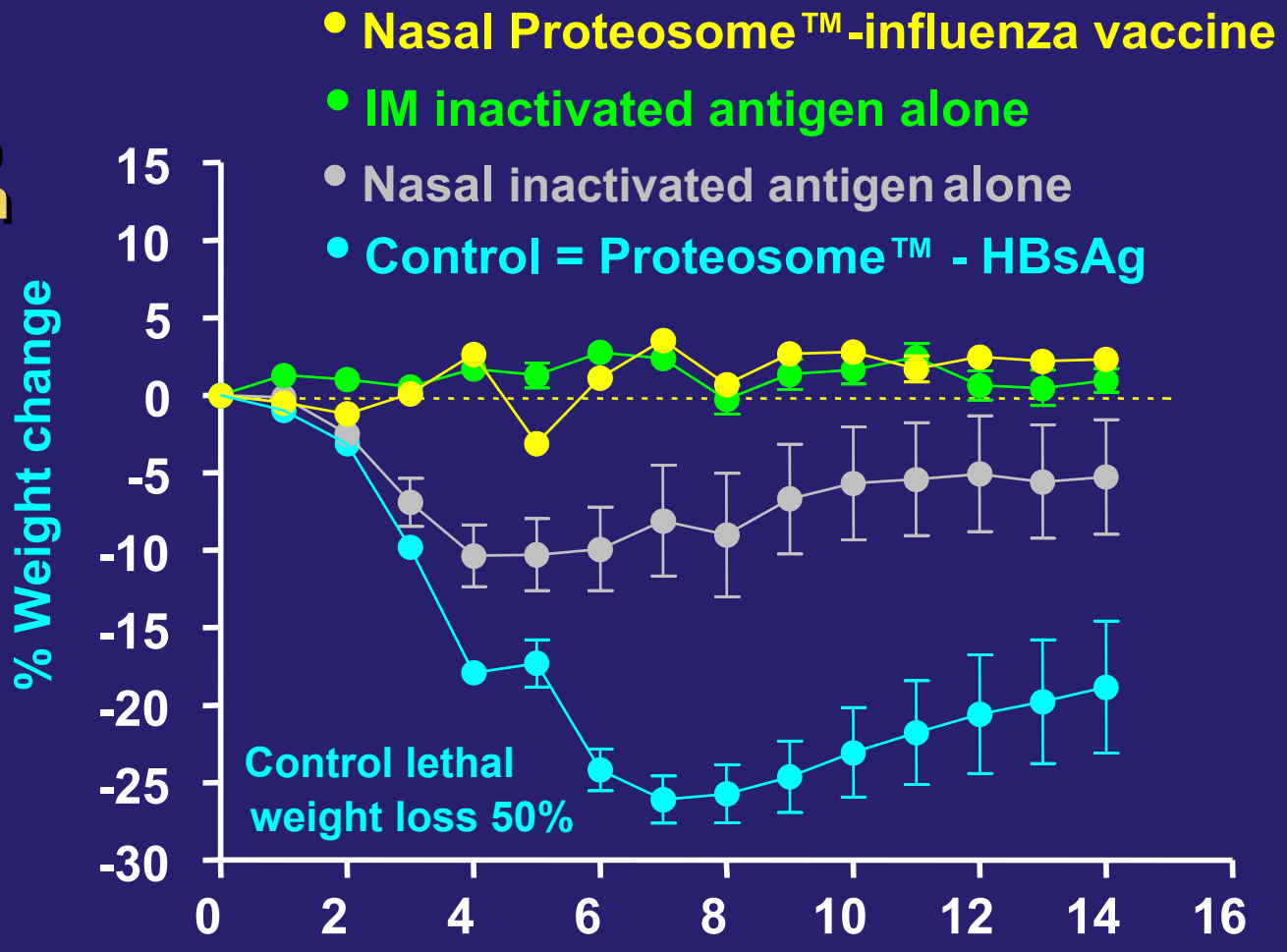


- Restimulated spleen cells from FluINsure-immunized mice yield 5 x greater IFN-γ response, but 40 x less IL-5, than spleen cells of mice that received IM or IN antigen alone (A/H1N1 data shown, others similar).



Intranasal FluINsure™
Protective Effects in Mice

- Monovalent vaccine followed by 4.0 LD_{50} nasal challenge with homologous mouse-adapted virus.
- Protection against lethality and weight loss equivalent to IM vaccine.



GLP Toxicity Data for Intranasal Proteosome™-Influenza Vaccines

- 400 mice in controlled studies with complete clinical and histopathologic evaluation
- No treatment-related clinical effects or weight loss
- No gross or histopathologic findings deemed treatment-related
  - Nasal and perinasal tissues
  - Respiratory tract
  - Olfactory bulb, other CNS tissues
  - Other tissues
- Ferrets receiving full human dose show no weight loss, clinical illness, or fever post-vaccine

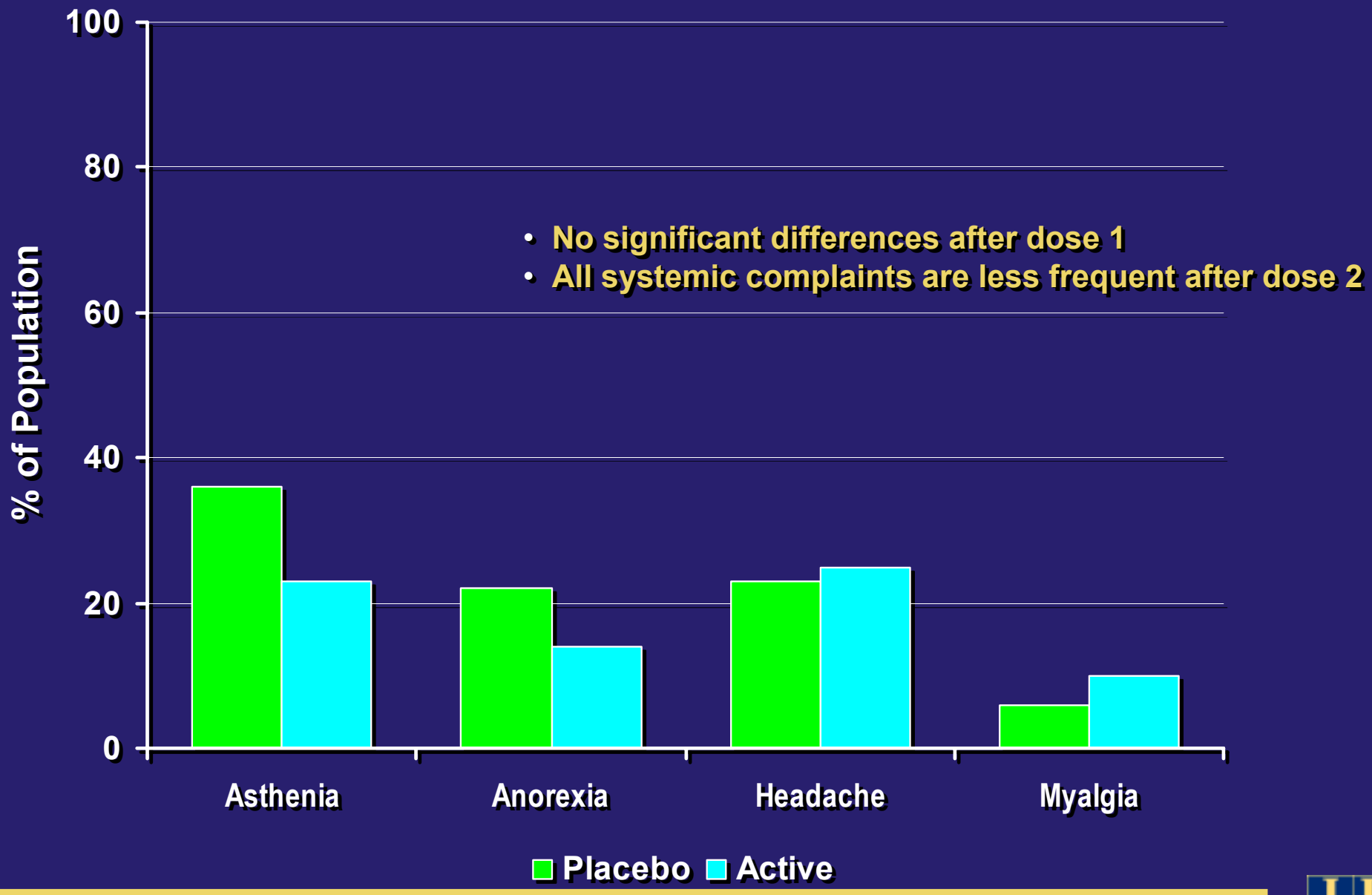
Clinical Studies of Proteosome™-Influenza Vaccines

Study Number	Vaccine	Population	Prior Immune Status	N	Study Status
0901	Monovalent prototype	18-45 y.o.	Seronegative (H1N1)	54	Complete
0902	Monovalent prototype	18-45 y.o.	Unselected, stratified on HAI	100	Complete
13001	Trivalent	18-50 y.o.	Unselected	78	Complete
13002	Trivalent (two-dose reg.)	18-50 y.o	Unselected	59	Complete
13003	Trivalent (one dose reg.)	18-50 y.o.	Unselected	40	Complete
13004	Trivalent, challenge	18-50 y.o.	Seronegative (H3N2)	75	Complete
13005	Trivalent, challenge	18-50 y.o.	Seronegative (H3N2)	102	Final data audits
				508	

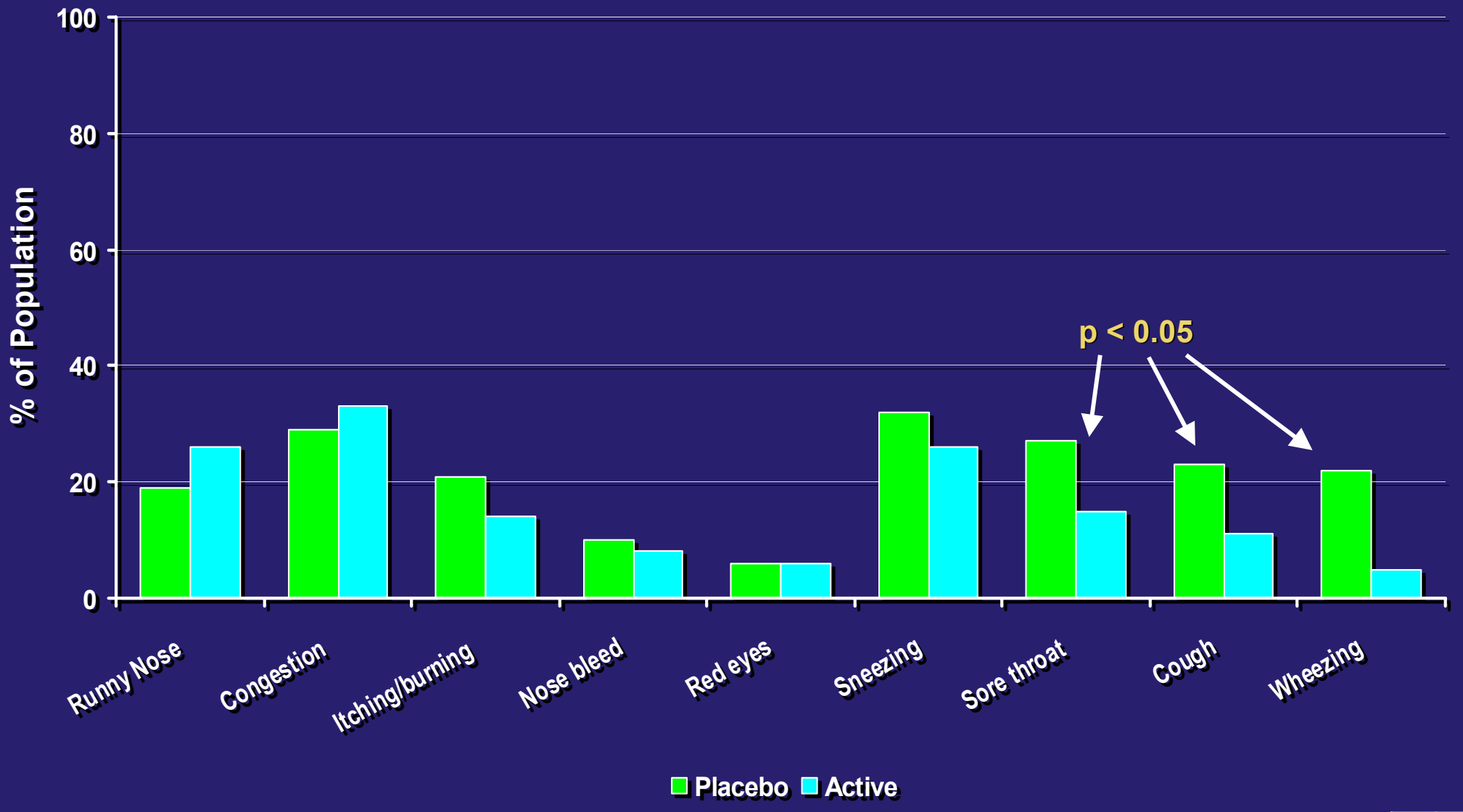
Safety Evaluation of FluINsure™

- Observation x 30 minutes after each dose
  - Vital signs
  - Immediate complaints actively solicited
- Seven-day reactogenicity evaluation after each exposure
  - Daily evening temperatures
  - Standard graded diary of local and systemic complaints
  - Repeated ENT exams
- Routine AEs through study termination
- CBC, serum chemistries, U/A before and after treatment course.

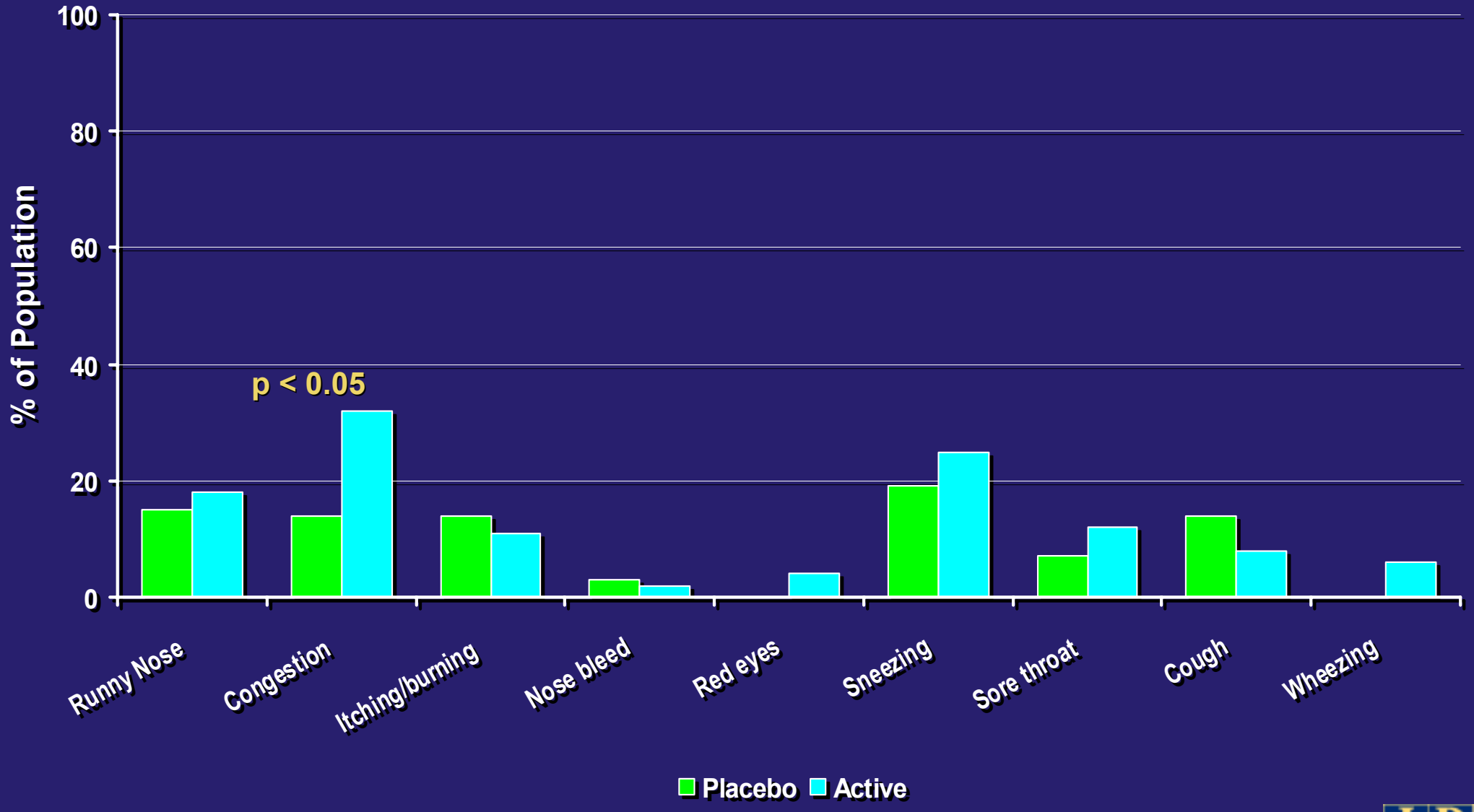
Systemic Complaints in 7 Days after FluINsure™ or Placebo Dose 1



Local/Respiratory Complaints in 7 Days after FluINsure™ or Placebo Dose 1



Local/Respiratory Complaints in 7 Days after FluINsure™ or Placebo Dose 2

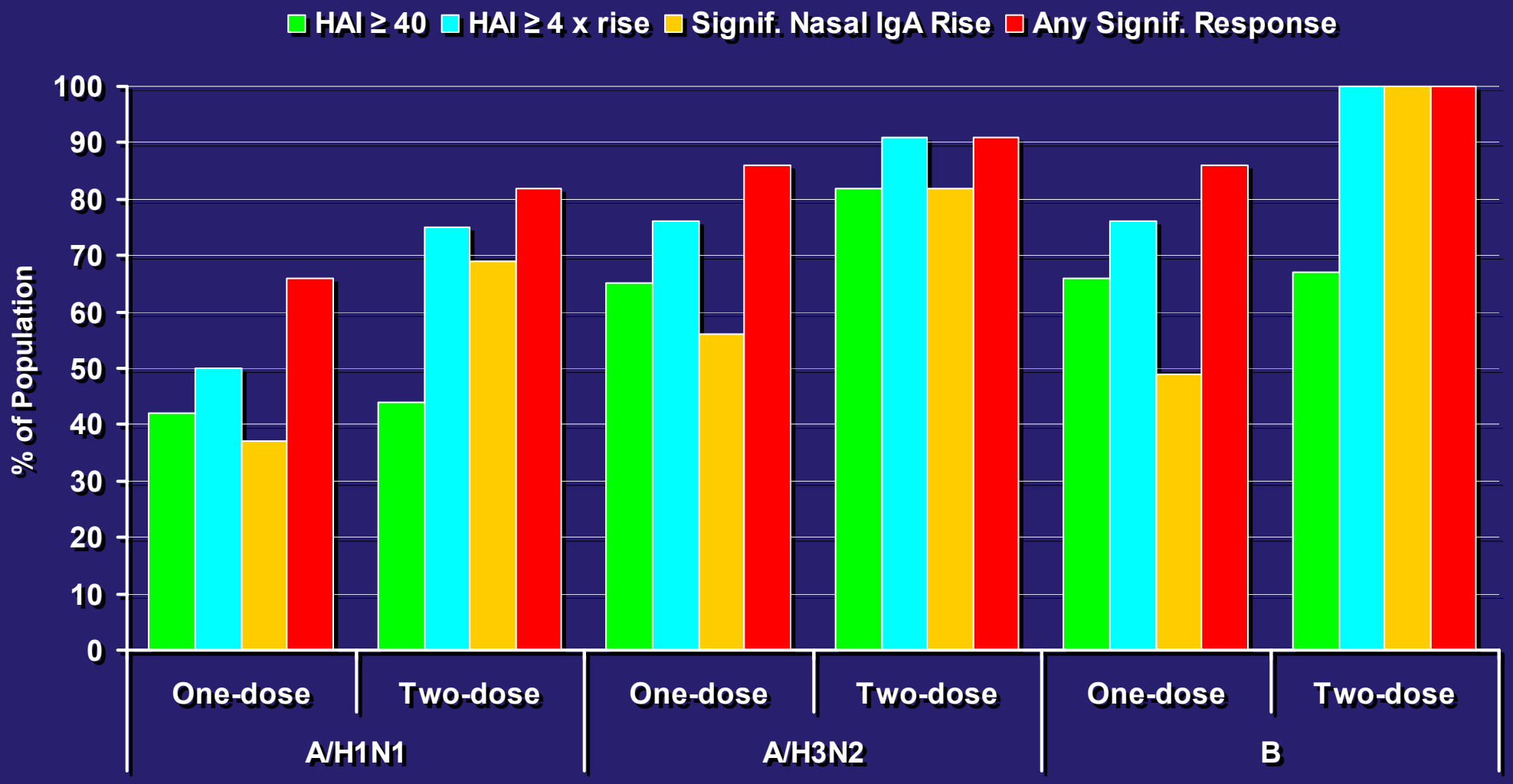


FluINsure™ Safety Summary

- Febrile responses (rare) and systemic complaints post-vaccine are not significantly associated with active product.
- 20 – 40% of recipients complain of rhinorrhea or congestion
  - > 90% mild (effect on daily activities nil)
  - Transient (median 2 days)
  - *Incidence generally not different than placebo*
  - No dose-response
- No association of AEs in any COSTART body system with active treatment.

Intranasal FluINsure™ Vaccine

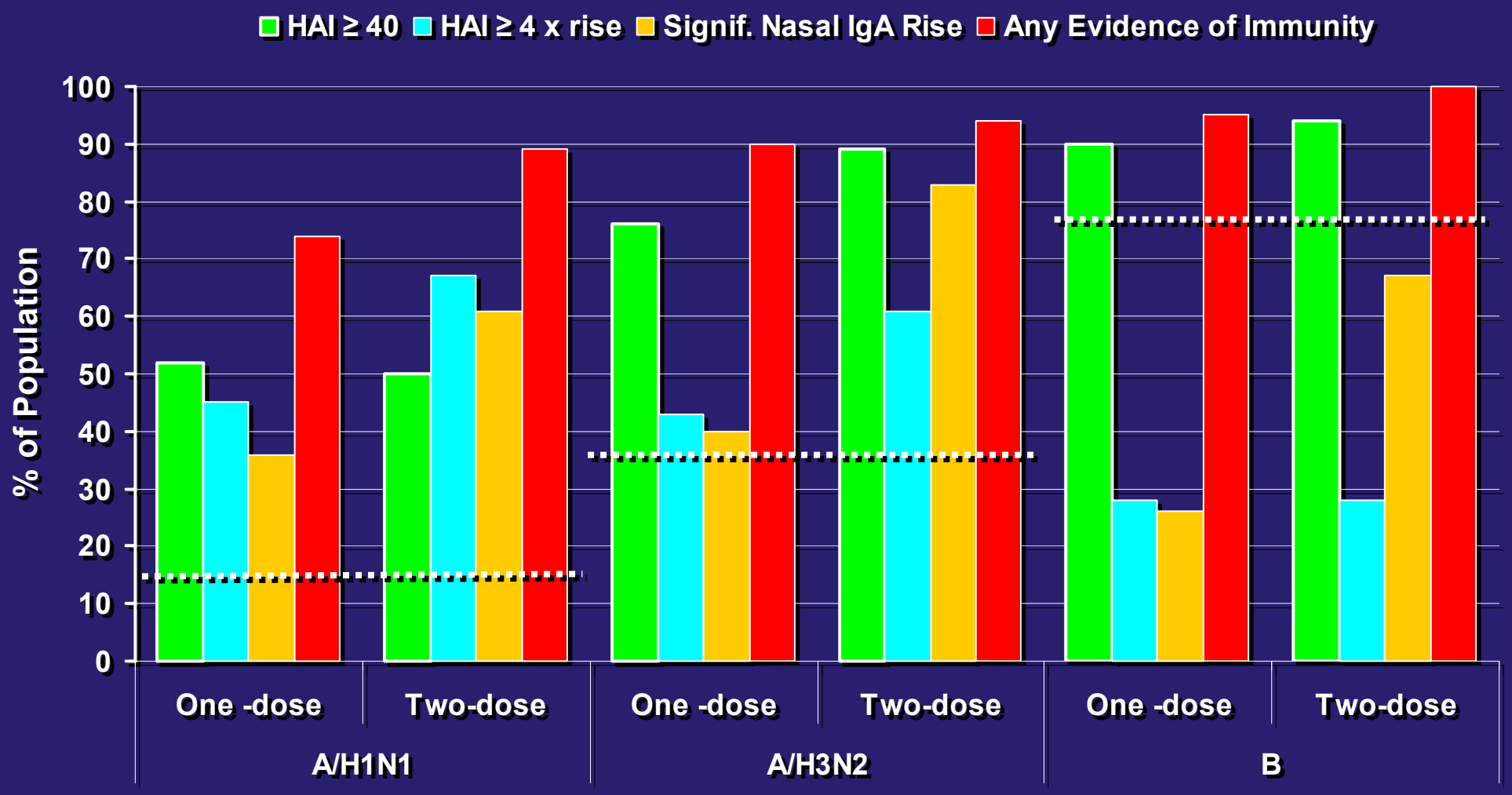
Immunogenicity in "Susceptible" Humans*



* "susceptibles" = persons with baseline serum HAI titers < 1:40 for relevant virus

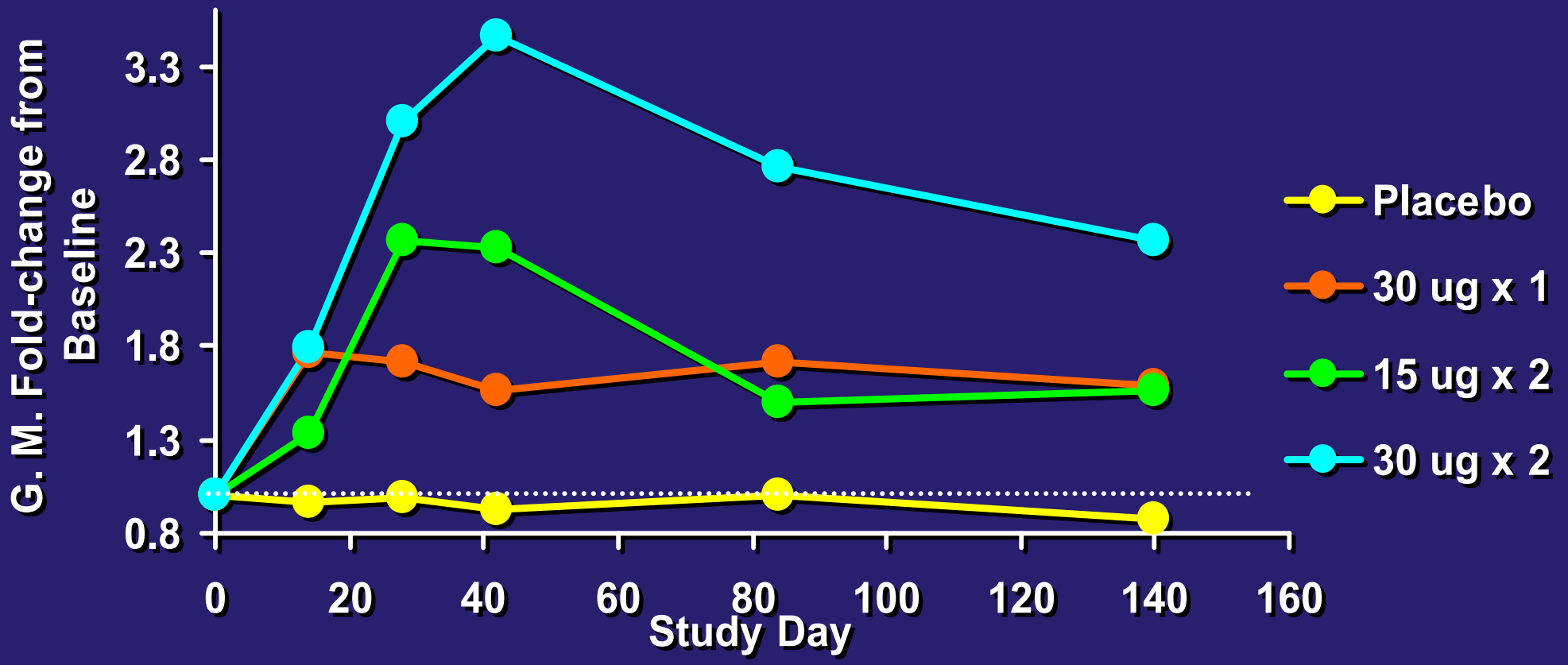
Intranasal FluINsure™ Vaccine

Immunogenicity in A General Population



..... Dotted lines represent frequency of pre-existing immunity (HAI ≥ 40) by virus subtype

Kinetics of the Nasal sIgA Response to FluINsure™; A/H1N1 Example



FluINsure™ in Challenge Studies

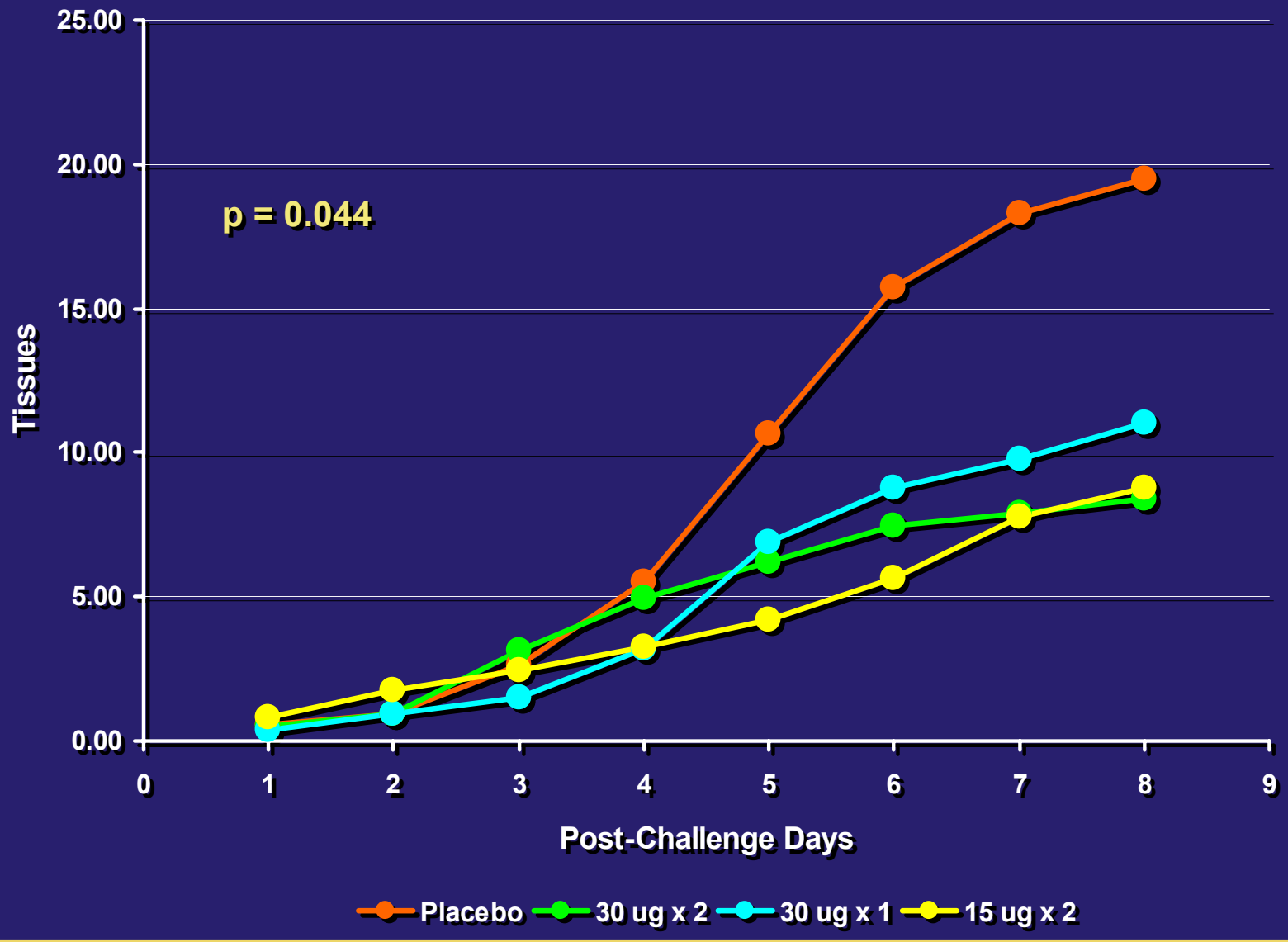
- 177 subjects with baseline serum reciprocal HAI titers ≤ 10 for A/Panama/2007/99 immunized with placebo, one dose (30 µg), or two doses (15 or 30 µg).
- On day 42, 67 subjects without confounding URI symptoms challenged IN with $10^{5.5}$ EID_{50} of A/Panama
- Seven day f/u for:
  - Fever, nasal discharge, otitis, pharyngitis
  - Cough, runny nose, congestion, sore throat, headache, myalgia, asthenia
  - Viral shedding by culture, antigen detection, and RT-PCR on daily nasal wash
- Post-challenge serum for seroconversion

Pooled Challenge Data to Date

	Febrile Illness	Any Systemic Illness	Any Illness + Lab Confirmation	Febrile Illness + Lab Confirmation
Placebo	20.0%	37.2%	44.4%	20.0%
One dose	7.0% 65% efficacy p = 0.07	16.3% 56.2% efficacy p = 0.034	23.3% 47.5% efficacy p = 0.044	7.0% 65% efficacy p = 0.07
Any two dose	0% 100% efficacy p < 0.001	8.8% 76.3% efficacy p = 0.001	7.0% 84.2% efficacy p < 0.001	0% 100% efficacy p < 0.001

- Tests of homogeneity indicate that data from two studies can be pooled.
  - Lower two-dose regimen actually sl. superior to the higher dose

Nasal Symptom Modulation: Cumulative Tissue Use

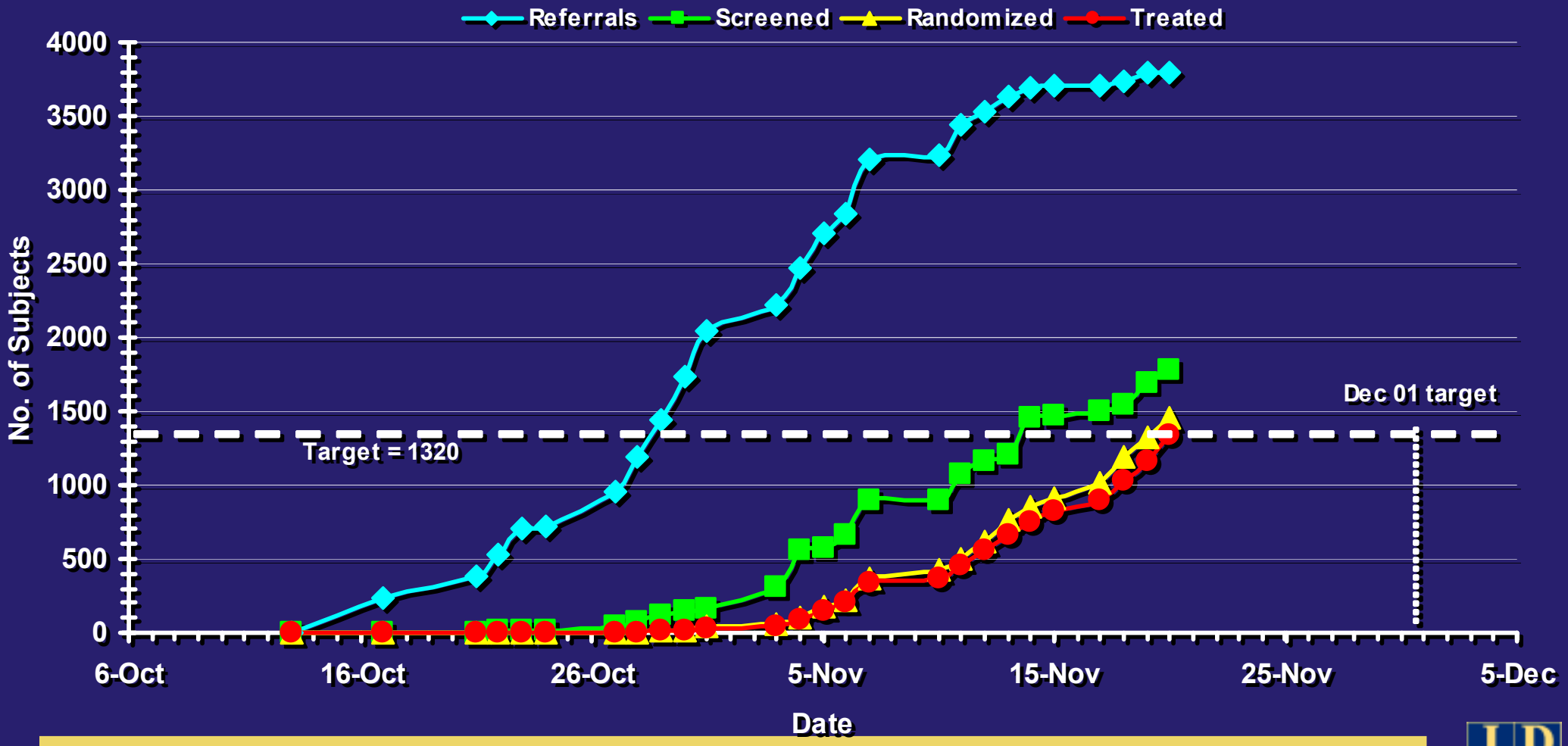


Challenge Study Results Summary

- The model is consistent in two iterations
- FluINsure is clearly active against:
  - Febrile illness
  - Systemic illness
  - Any illness
  - Febrile illness with confirmed infection
  - Any illness with confirmed infection
- Two-dose regimens highly active, regardless of dose level
- One-dose regimen has somewhat smaller effects, but appears active on all endpoints and impacts nasal symptoms.

Where is FluINsure™ Now?

- Enrolled 1,345 subjects to assess field efficacy of 1 and 2 dose regimens
- Bridging to final formulation and delivery configuration, first pediatric studies, in 2004
- Pivotal efficacy Q4 ’04 or summer ’05 S. hemisphere



Nasal Plague Vaccine

Protollin™ Adjuvant Technology

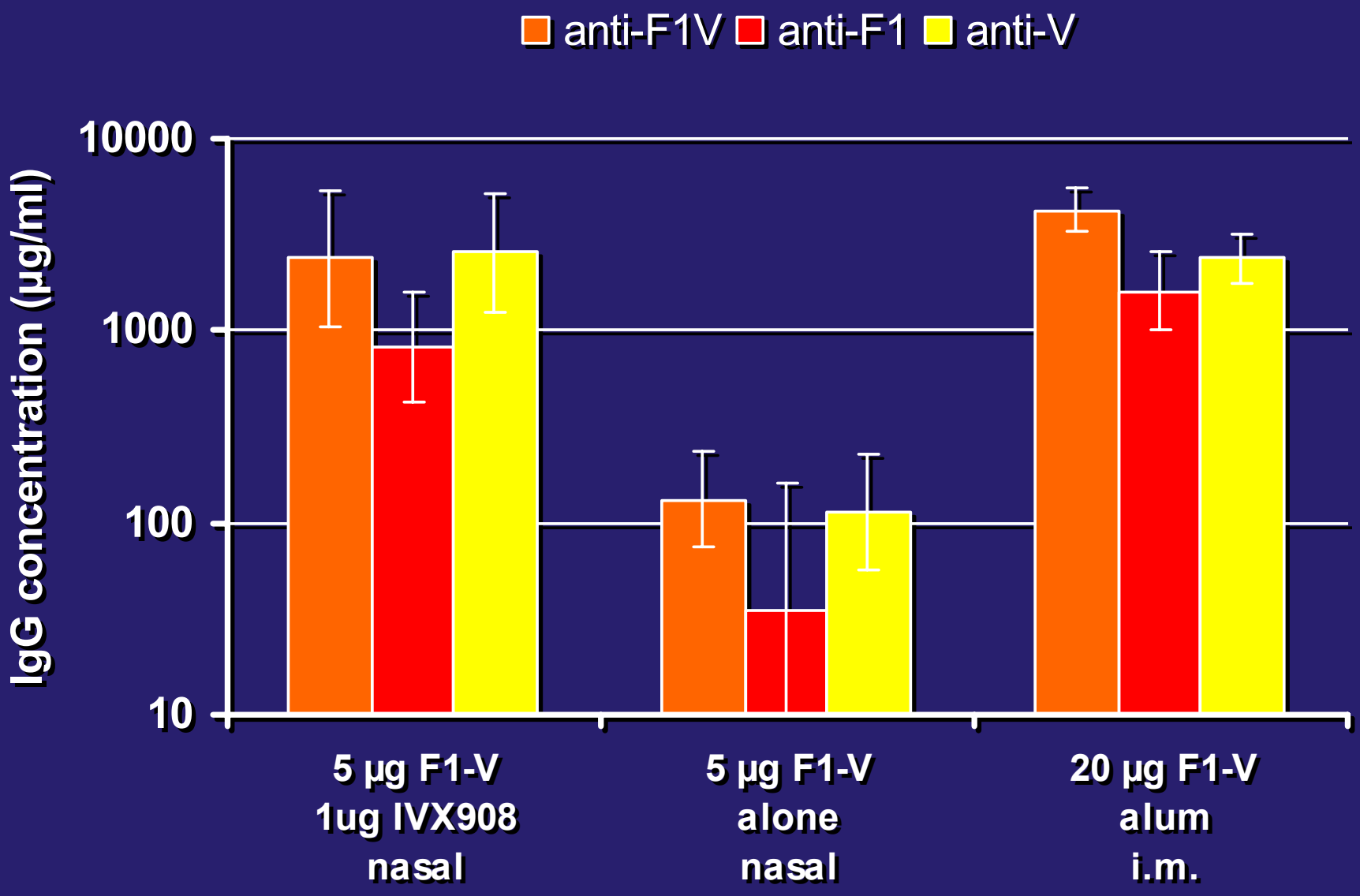
Protollin™

- Nasal adjuvant which is active with simple mixing.
- Suitable for wide variety of antigens, including completely hydrophilic recombinant molecules.
- Combines immunostimulatory activities of Proteosomes and LPS.
- GLP preclinical safety in almost 1,000 animals, including outbred and inbred strains.
- Human clinical experience in > 130 subjects at nasal doses up to 1.5 mg.

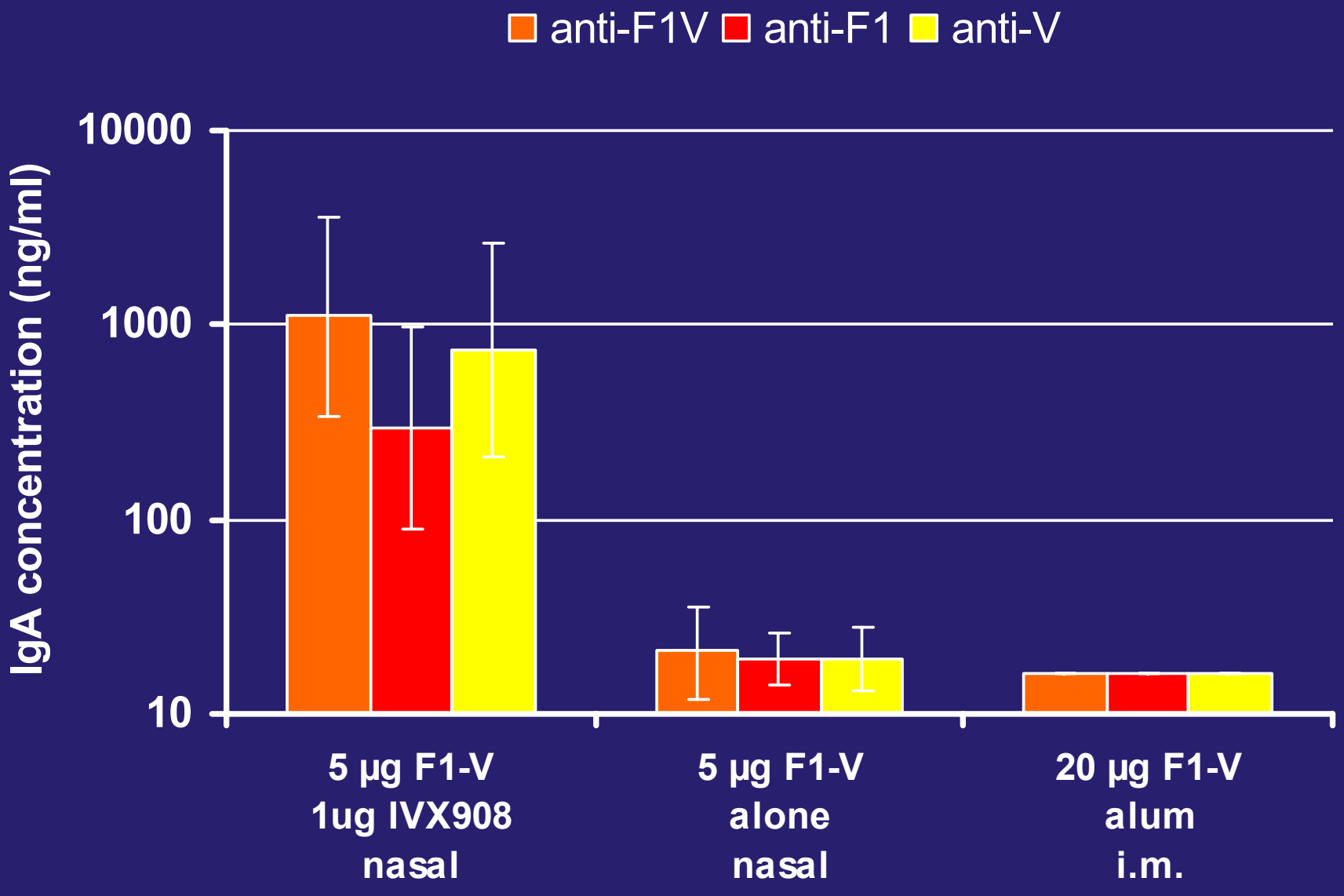
Plague Targets

- Plague vaccine development currently focuses on:
  - F1, a putatively antiphagocytic surface antigen
  - V, a protein found in all virulent strains which modulates host cytokine responses
- Parenteral immunization with F1, V, or the fusion protein F1V absorbed to alum can protect mice against high-dose aerosol challenge with *Y. pestis*, but is not adequate in NHPs. ? desirability of "local" immunity.

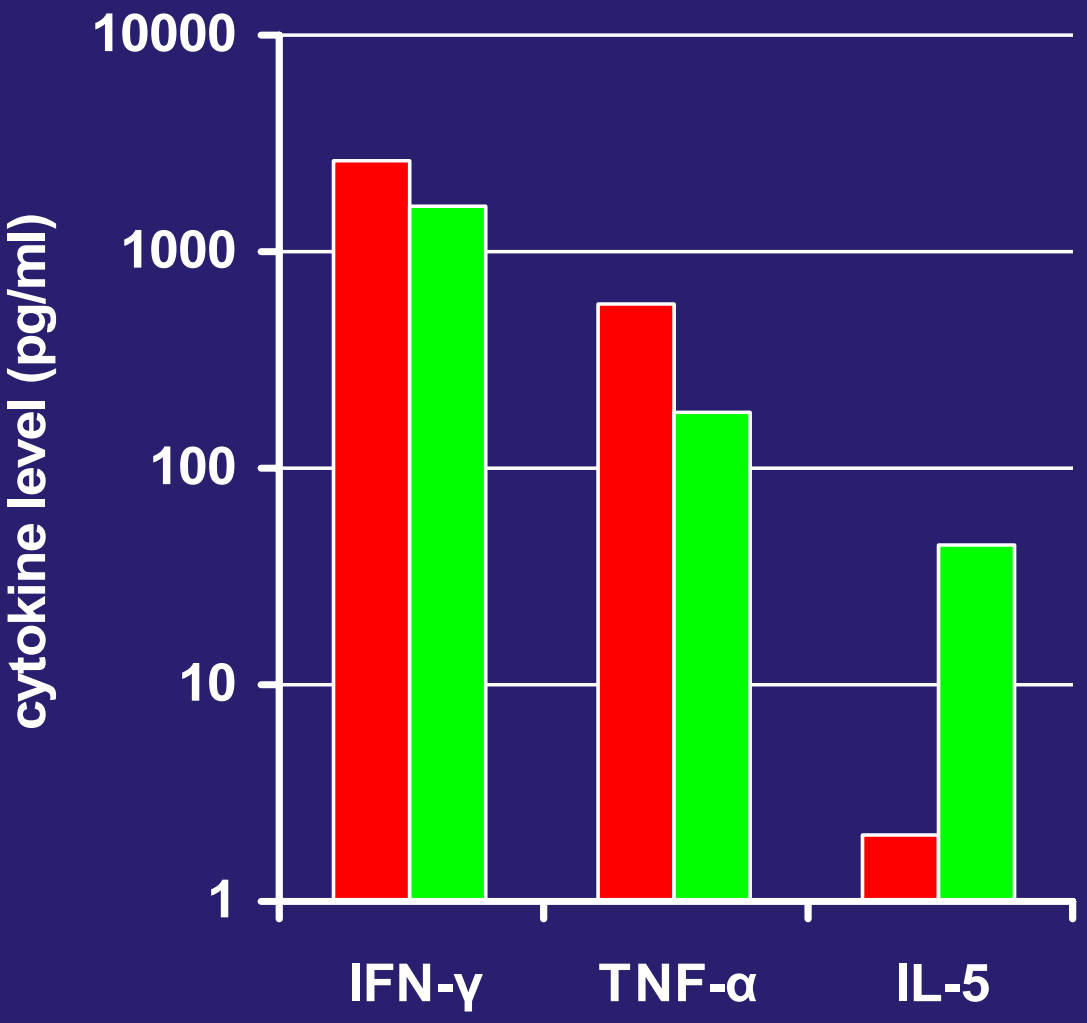
Mouse Serum IgG Responses to Nasal Protollin™-F1V



Mouse Lung Wash IgA Responses to Nasal Protollin™-F1V



Th1-shifted Cytokine Pattern Elaborated by Restimulated Spleen Cells after Nasal Protollin™-F1V



F1V + IVX908 nasal

IFN-γ / IL5 ratio = 1300
TNF-α / IL5 ratio = 290

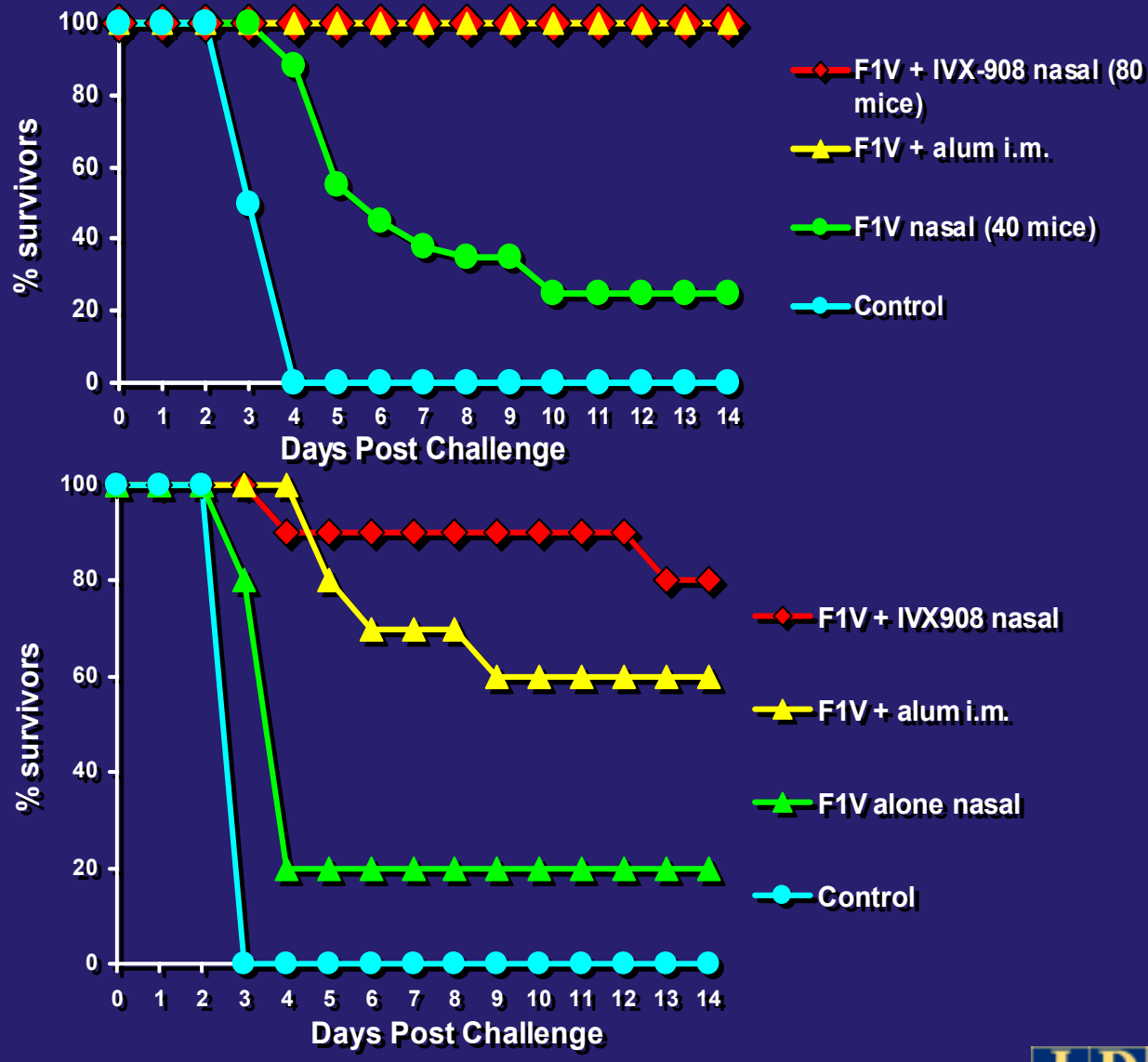
F1V + alum i.m.

IFN-γ / IL5 ratio = 36
TNF-α / IL5 ratio = 4

Murine Challenge Protection by Protollin™ F1V

- Protection provided by nasal Protollin-F1V is similar to IM F1V + alum in 169 LD_{50} aerosol challenge, but

- May be superior in more stringent (> 250 LD_{50}) model



Protollin™

- A widely-applicable "just mix it" nasal adjuvant with a substantive pre-clinical and human safety experience.
- In the plague system, nasal Protollin™-F1V
  - Induces F1 and V serum responses equivalent to IM vaccine
  - Induces superior local IgA responses
  - Induces a Th1 cytokine profile
  - Provides at least competitive protection
  - Is a candidate for NHP protection testing
- Protollin™ is an attractive candidate for other nasal antigens.