

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Treanor JJ, Campbell JD, Zangwill KM, et al. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N Engl J Med* 2006;354:1343-51.

Supplementary Materials

Enrollment Criteria

Subjects were carefully screened for health status prior to enrollment. The specific entry criteria follows, and are included as a supplementary appendix. Specifically, subjects were determined to be in good health, as determined by vital signs (heart rate, blood pressure, oral temperature), medical history and a targeted physical examination based on medical history, prior to enrollment, and in Stage I have normal laboratory values of Hgb, WBC, Plt, ALT, and creatinine prior to the first immunization. Women of childbearing potential were required to have a negative urine pregnancy test on the day of immunization and to agree to practice adequate contraception (ie, barrier method, abstinence, and licensed hormonal methods) for the entire study period. Subjects were excluded from participation if they had a known allergy to eggs or other components of the vaccine, were undergoing immunosuppression as a result of an underlying illness or treatment, had an active neoplastic disease or a history of any hematologic malignancy, were using oral or parenteral steroids, high-dose inhaled steroids (>800 mcg/day of beclomethasone dipropionate or equivalent) or other immunosuppressive or cytotoxic drugs, had a history of receiving immunoglobulin or other blood product within the 3 months prior to enrollment in this study, had received any other licensed vaccines within 2 weeks (for inactivated vaccines) or 4 weeks (for live vaccines) prior to enrollment in this study, had an acute or chronic medical condition that, in the opinion of the investigator, would render vaccination unsafe or would interfere with the evaluation of responses (this includes, but is not limited to: known chronic liver disease, significant renal disease, unstable or progressive neurological disorders, diabetes mellitus, and transplant recipients), any history of severe reactions following immunization with contemporary influenza virus vaccines, any an acute illness, including an oral temperature greater than 100.4 F, within 1 week of vaccination, if they had received an experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 1 month prior to enrollment in this study, or expects to receive an experimental agent during the 7-month study period, or if they had any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

Unsolicited and unassociated adverse events

The most common unsolicited symptoms reported in the 28 days following each vaccination were gastrointestinal symptoms (nausea, vomiting, diarrhea, or gastroenteritis) reported in 21 subjects after dose 1 and 7 subjects after dose 2, and upper respiratory tract symptoms (rhinorrhea, sore throat, cough, or URI) reported in 30 subjects after dose 1 and 25 subjects after dose 2. There were no significant differences in the frequency of these reports between any of the dose groups and placebo.

Clinical laboratory safety testing done on subjects in stage 1 did not reveal clinically significant abnormalities. Changes were noted in 24 subjects following dose 1 and in 11 subjects following dose 2; these included decreases in hemoglobin in 19 subjects (minimum observed value 11.1 g/dL in a female subject; maximum decrease from baseline 1.9 g/dL in an additional female subject), increases in ALT in 2 subjects (maximum observed value 232 IU/L, falling to 66 IU/L on repeat testing), changes in peripheral white blood cell count in 6 subjects (4 with slight decrease, minimum value $3.1 \times 10^3/\mu\text{L}$, and 2 with slight increase, maximum value $11.9 \times 10^3/\mu\text{L}$), and slightly

increased serum creatinine (to 1.3 g/dL) in 1 subject. There was no association of these abnormalities with dose of vaccine received, and none of the laboratory abnormalities were associated with symptoms.

There was one serious adverse event in the study, which was judged by the investigator to be unrelated to the vaccine. A 52 year-old male participant in stage II was found dead at home 24 days after receipt of vaccine dose 1. He had reported mild pain at the injection site on day 1 and no other symptoms on the 7-day memory aid. He had been afebrile after vaccination and reported no concomitant medication use.

The subject had been observed to be in ill health by his landlord on the day of death and was found dead later on the same day. The landlord and several other acquaintances and family members reported that the subject had engaged in very heavy bouts of binge drinking in the two weeks prior to his death, and that large numbers of open containers of alcohol were observed in his apartment. Autopsy revealed extensive bruising consistent with falls, and marked steatosis of the liver. The heart was normal, without evidence of myocarditis or infarction. The cause of death was determined by the medical examiner to be due to chronic alcoholism.

Supplementary Table 1. Demographics of enrolled subjects

Characteristic	Number of subjects (%) in the following dose groups				
	Placebo	7.5 mcg	15 mcg	45 mcg	90 mcg
White	39 (81.3)	81 (80.2)	78 (77.2)	75 (76.5)	84 (81.6)
Black	3 (6.3)	5 (5.0)	6 (5.9)	12 (12.2)	11 (10.7)
Asian	4 (8.3)	13 (12.9)	13 (12.9)	10 (10.2)	8 (7.8)
Pacific Islander	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)
Multiracial	2 (4.2)	2 (2.0)	2 (2.0)	1 (1.0)	0 (0.0)
Hispanic	4 (8.3)	13 (12.9)	11 (10.9)	6 (6.1)	13 (12.6)
Female	22 (45.8)	51 (50.5)	65 (64.4)	42 (42.9)	55 (53.4)
Previous vaccine	20 (41.7)	39 (38.6)	42 (41.6)	42 (42.9)	44 (42.7)
Age (median, range)	38 (21-62)	39 (18-64)	40 (22-64)	38 (19-63)	38 (18-64)

