Development of an Influenza HA-flagellin fusion vaccine with improved safety and immune response

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Abstract

Introduction: We evaluated 3 novel influenza vaccine constructs consisting of the globular head of the HA1 domain of the A/California/07, Novel H1N1 (VAX128) genetically fused to the TLR5 ligand, flagellin, and produced in *E. coli*. HA1 was fused to the C-terminus of flagellin in VAX128A, replaced the D3 domain of flagellin in VAX128B and was fused in both positions in VAX128C.

Methods: 112 healthy subjects 18-49 and 100 adults ≥ 65 years old were enrolled in a double blind, placebo controlled clinical trial conducted at two centers. Vaccines were administered IM at doses ranging from 0.5-20 µg. A phase 2 study was performed in 100 subjects 18-64 years old comparing 1.25 and 2.5 µg doses. All subjects were followed for safety and sera collected pre- and post-vaccination were tested by the hemagglutination-inhibition (HAI) assay. Serum C-reactive protein, cytokine levels and anti-flagellin antibody were also measured.

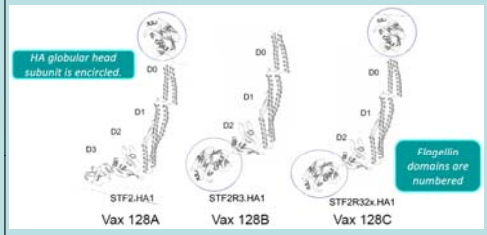
Results: In adults 18-49 years old, serum HAI antibody responses were seen after doses as low as 0.5 µg. Doses of 1.25 to 2.5 µg induced a GMT of 1:250 with over 90% seroconversion and seroprotection. In young adults, VAX128C was safe at 20 µg, the highest dose tested. VAX128A was tolerated up to 8 µg for and VAX128B up to 16 µg. Cytokine release was observed with VAX128B at a dose 16 µg but was not observed with VAX128C at 20 µg. In adults ≥65 years, a similar safety pattern was observed, however doses of 4-8 µg were needed to induce a ≥4-fold rise in HAI titer, 50% seroconversion and 70% seroprotection.

Discussion: The globular head of the influenza HA expressed in a prokaryotic system was able to induce a functional antibody response at low doses. Altering the configuration of the HA1 and flagellin produced influenza vaccines with a larger safety window than the VAX128A prototype. VAX128C was well tolerated at the highest dose tested and was highly immunogenic. These data indicate that flagellin adjuvanted vaccines can be designed to minimize systemic reactogenicity and still retain strong immunogenicity.

A Modern Influenza Vaccine

- Our laboratory has developed a method for making properly-folded hemagglutinin globular head in *E. coli.* Approximately 260 amino acids of HA are fused genetically to flagellin, the ligand for Toll-Like Receptor 5.
- This creates a single chimeric protein comprising an antigen and an immunopotentiating signal. This type of vaccine protects mice and ferrets from death and disease. VAX128 contains the globular head of the H1N1 California 07 HA fused to flagellin from *Salmonella typhimurium*.

Influenza Vaccine
HA Subunit Vaccine Design



Background

- The worldwide need for seasonal and pandemic influenza vaccines requires innovative technologies to enhance the immunopotency of influenza vaccines and to increase vaccine production.
- We developed a novel H1N1 pandemic influenza vaccine designated, VAX128. VAX128 is a recombinantly produced vaccine that activates the immune response by coupling a potent immune stimulator, the bacterial protein flagellin (STF2), to the globular head domain of the influenza HA antigen.
- The globular head domain of HA stably refolds to form the conformationally sensitive antigenic sites that span the majority of the neutralizing epitopes in HA. VAX128 is highly protective in animal models.
- The influenza A H1 strain California (CA07), the cause of the 2009-2010 pandemic and now a component of the 2010- 2011 seasonal trivalent influenza vaccine, was selected as the prototype strain for Phase I clinical testing.
- The primary objective of this initial study is to find a safe and immunogenic construct and dose by comparing three different configurations of VAX128, which are referred to as "VAX128A", "VAX128B" and "VAX128C".

Study Design

- Dose escalation study in healthy men and women age 18-49 years and ≥ 65 in first study and dose confirmation in healthy subjects 18-64 years.
- All doses formulated at vaccine site and given as a single 0.5 ml IM injection. Vaccine buffer was used as the placebo control.
- Serum specimens for cytokines 2 hrs and for CRP 24 hrs post-vaccination
- Safety recorded for first 7 days by clinic visit and diary
- Immune response (HAI) measured on day 0, 7, 14 and 28

RESULTS

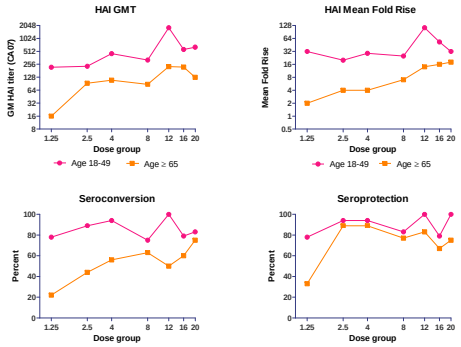
Number of subjects enrolled at each dose level by VAX128 vaccine construct A, B or C or placebo

Dose (µg)	Age 18-49 years VAX128 A	B	C	Plac.	Total	Age ≥ 65 years VAX128 A	B	C	Plac.	Total	Age 18-64 VAX128C Total
0				10	10	0			10	10	
0.5	3	3	3	0	9	0	0	0	0	0	
1.25	3	3	3	0	9	3	3	3	0	9	49
2.5	12	3	3	0	18	3	3	3	0	9	51
4	12	3	3	0	18	3	3	3	0	9	
8	6	3	3	0	12	21	6	3	0	30	
12	0	3	3	0	6	0	3	3	0	6	
16	0	12	12	0	24	0	12	3	0	15	
20	0	0	6	0	6	0	0	12	0	12	
Total	36	30	36	10	112	30	30	30	10	100	100

Demographic characteristics of 312 subjects who received one dose VAX128 or placebo

	Study 1, age 18-49 years VAX128 construct A	B	C	Plac.	Study 1, age ≥ 65 years VAX128 construct A	B	C	Plac.	Study 2, age 18-64 VAX128C dose 1.25 µg	2.5 µg
N	36	30	36	10	30	30	30	10	49	51
Age mean	33.3	31.2	31.2	26.8	71.7	74	73.7	72.6	39.4	36.3
Male %	50	63	44	40	37	37	33	40	41	45
White %	61	57	53	50	100	97	97	100	78	82
Black %	39	43	47	40	0	3	0	0	22	18
Hispanic %	53	47	42	50	0	0	3	0	39	41

Comparison of immune response by dose among 112 subjects 18-49 years old and 100 subjects ≥ 65 years old who received a single IM dose of VAX128



Percent of subjects 18-49 with injection site and systemic symptoms in VAX128-01, n=112

Vaccine construct	Dose in µg	No. of subjects	Injection site Mild/ Mod	Injection site Severe	Systemic Mild/ Mod	Systemic Severe
VAX128A	0.5-1.25	6	83	17	50	0
	2.5	12	83	0	25	0
	4	12	67	8	0	8
	8	6	83	17	50	0
VAX128B	0.5-12	18	83	0	39	0
VAX128C	0.5-12	18	72	6	33	0
VAX128B	16	12	83	0	17	8
VAX128C	16-20	18	72	17	33	6
Placebo	0	10	20	0	40	0

Number (%) of subjects who had a 4-fold increase in cytokines after VAX128

VAX128 construct/dose (µg)	B/16 n=12 n, (%)	C/16-20 n=18 n, (%)
No. with systemic symptoms (SS)	1 (8.3)	1 (5.6)
No. SS and inc. in CRP or IL-6	1 (8.3)	0
No. ≥ 4-fold increase in CRP	5 (41.7)	0
No. ≥ 4-fold increase in IL-6	5 (33.3)	1 (5.6)

Cytokine and CRP Response in young adults who received high dose VAX128B

Dose (µg)	CRP D0	CRP D1	TNF Pre	TNF 2 hr	IL-6 Pre	IL-6 2 hr	Symptom grade Local	Symptom grade Sys.
8	17	51	0	26	2	132	1	0
16	13	100	1	12	2	20	1	1
16	2	44	1	2	2	16	1	0
16	3	68	0	250	2	832	2	0
16	1	24	2	68	2	1772	0	0
16	2	59	3	835	3	7488	2	3

VAX128-02, Phase 2 Dose Confirming Study

- 100 subjects 18-64 years old
- Randomized subjects to 1.25 and 2.5 µg doses of VAX128
- Two clinical sites
- Safety evaluation
- Cytokine at 2 hrs
- Serum HAI evaluation on days 0 and 21

Serum HAI response in subjects 18-64 years after one i.m. injection of VAX128C in study 2

	VAX128C dose (µg) 1.25 (n=47)	95% C.I.	2.5 (n=50)	95% C.I.	Total (n=97)	95% C.I.
GMT Day 0	16	11-23	26	15-43	20	14-28
GMT Day 21	306	187-502	478	290-789	385	272-546
Mean fold response	20	12-33	19	11-31	19	13-27
Seroconversion	79%	64-89	80%	66-90	79%	70-87
Seroprotection	89%	77-97	94%	94-99	92%	84-96

❖ *Seroprotection (minimum post-vaccine titer of 1:40)*
❖ *Seroconversion (increase in HI titer at least 4 fold with a minimum titer of 40)*

Number (%) of subjects 18-64 with local and systemic symptoms in study 2

	Dose ranges (µg) 1.25 n=49 (%)	2.5 n=51 (%)	Total n=100
Local			
None	9 (18)	7 (14)	16
Mild	26 (53)	27 (53)	53
Moderate	12 (24)	16 (31)	28
Severe	2 (4)	1 (2)	3
Systemic			
None	40 (82)	38 (75)	78
Mild	3 (6)	9 (18)	12
Moderate	6 (12)	4 (8)	10
Severe	0	0	0

Conclusions

- The HA globular head can be stably expressed in a prokaryotic (E. coli) system positioned in either the C terminal or D3 position of flagellin.
- We found that placing the HA antigen in the R3 position in VAX128B and VAX128C produced a vaccine that was as immunogenic and better tolerated at higher doses than the C terminal fusion construct (VAX128A).
- VAX128B and VAX128C were well tolerated at 16 and 20 µg, respectively, providing the necessary safety window important when constructing the multivalent seasonal influenza vaccine.
- If the 1-2 µg level is immunogenic, then it would be possible to produce a trivalent or quadrivalent vaccine that is well within the safety window.

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