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The Increase of the Magnitude of Spontaneous Viral Blips in Some Participants of Phase II Clinical Trial of Therapeutic Optimized HIV DNA Vaccine Candidate

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Abstract: We developed a candidate DNA vaccine "DNA-4" consisting of 4 plasmid DNAs encoding Nef, Gag, Pol(rt) and gp140 HIV-1 proteins. The vaccine was found to be safe and immunogenic in phase I clinical trial. Here we present the results of phase II clinical trial of "DNA-4". This was a multicenter, double-blind, placebo-controlled clinical trial of safety, immunogenicity and dose selection of "DNA-4" in HIV-1 infected people receiving antiretroviral therapy (ART). 54 patients were randomized into 3 groups (17 patient - group DNA-4 0.25 mg, 17 patient - group DNA-4 0.5 mg, 20 patient - group placebo). All patients were immunized 4 times at days 0, 7,11,15 followed by 24 weeks follow-up period. “DNA-4” was established as safe and well tolerated at doses 0.25 mg and 0.5 mg. We obtained ~~the~~ data on spontaneous viral ~~blips magnitude~~ increases that suggest the possible influence of therapeutic DNA vaccination on viral reservoirs in some patients on ART. We hypothesize that this influence was associated with the reactivation of proviral genomes.

Keywords: HIV; AIDS; DNA vaccine; clinical trial; therapeutic vaccine

1. Introduction

Since AIDS was ~~discovered~~ first described in 1981 about 60 million people have been infected with HIV, and about 30 million died of AIDS. In Russia more than 1.2 million infected individuals (59 per 100 000 of citizens) have been detected [1]. Despite great success in the field of antiretroviral therapy (ART), the pandemic of HIV infection is not yet contained. The development of vaccines against HIV/AIDS, both preventive and therapeutic, is the necessary step to stop further spread of the epidemic.

Our group has developed the candidate DNA vaccine "DNA-4" which consists of 4 plasmid DNAs encoding Nef, Gag, Pol(rt) and gp140 HIV-1 proteins of Eastern European subtype A. The candidate vaccine has passed preclinical studies in laboratory animals [2] and phase I clinical trial in healthy volunteers [3] . The vaccine was found to be safe and well-tolerated. The intramuscular immunization with "DNA-4" induced the development of HIV-specific mostly cellular immune responses in all trial participants. Some of induced immune reactions, e.g. TNFα, were similar to the reactions discovered in exposed seronegative individuals, who remain HIV uninfected despite repeated unprotected exposure to the virus [3, 4].

Here we present the results of phase II clinical trial of the candidate vaccine "DNA-4" in HIV-1 infected people receiving ART. The trial was conducted to assess safety and to determine an optimal dose of the vaccine for HIV-positive patient~~s~~. In the study, the "DNA-4" candidate vaccine was found

needs a better description: possibly “spontaneous viral blips, or transient increases in viral load, were…”

to be safe. In some trial participants the increase of the magnitude of spontaneous viral blips was demonstrated.

2. Materials and Methods

2.1. Study vaccine.

A candidate DNA-vaccine "DNA-4" has been developed in the Biomedical Center (St. Petersburg, Russia) in collaboration with Research Institute of Ultra Pure Biologicals (St. Petersburg, Russia). The vaccine contains four plasmid DNA encoding consensus sequences of *nef, gag, rt* or *gp140* HIV-1 FSU subtype A genes [2]. DNA-4 was manufactured by production facility of Research Institute of Ultra Pure Biologicals (St. Petersburg, Russia) in accordance with the existing Russian federal regulations. The plasmids were equally formulated in 1 ml of sterile saline solution with overall plasmid concentration of 0.25 mg/ml. Placebo vials contained 1 mL of saline solution without plasmids.

2.2. Phase II clinical trial design

Phase II clinical trial was a multicenter, double-blind, placebo-controlled study. It was conducted to assess the safety of two "DNA-4" doses (0.5 mg and 0.25 mg) in patients with HIV-1 receiving ART by the analysis of frequency and severity of adverse events.

The study was conducted in 7 Centers for the Prevention and Control of AIDS and Infectious Diseases situated in different Russian cities: Moscow region, Kazan, Tolyatti, Volgograd, Lipetsk, Kaluga, Izhevsk. All trial participants have been randomized into three equal groups and vaccinated four times with corresponding dose (0.5 mg or 0.25 mg or placebo) at days 1, 7, 11, and 15 with 22-weeks follow-up period. Candidate vaccine was administered intramuscularly in 1 mL of sterile saline solution in the deltoid muscle of each shoulder. Figure 1 shows the clinical trial design.

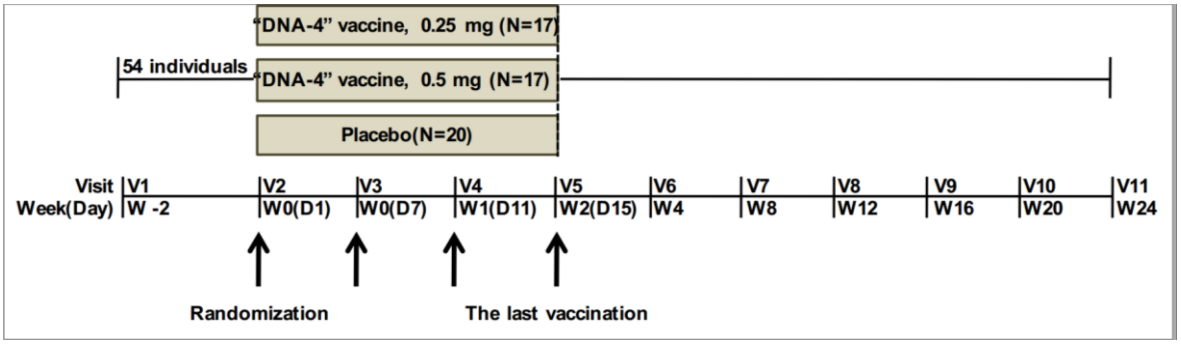


Figure 1. Clinical trial scheme. Arrows - days of immunization. .

Safety and tolerability were evaluated by the frequency and severity of adverse events (AE) according to subjective complaints, vital signs, physical examination, laboratory tests and development of local reactions. The severity of AE was assigned in accordance with the DAIDS scale, Version 1.0, December 2004. Each adverse event were raided by 4-grade scale: 1 - mild, 2 - moderate, 3 - severe, 4 - potentially life threatening.

Viral load was assessed at visits 2 and 6-11 by real-time PCR analysis. "AmpliSense HIV-Monitor-M-FL" kit (Russia) were used to detect transient viral increases above 50 copies/mL (sensitivity of the kit was 20 copies/mL).

Quantity and ratio of CD4 and CD8 T cells were measured by flow cytometry analysis.

2.3. Ethical compliance

The study was reviewed and approved by the Ethical Committee of the Ministry of Health of the Russian Federation (clinical trial approval number 222 from April 22, 2014). Volunteers provided

written informed consent following protocol review, and discussion and counseling with the clinical study team.

3. Results

3.1. Adherence and tolerability

54 HIV-1 infected individuals receiving ART participated in the study. All participants were randomized into three equivalent groups: 0.5 mg of vaccine - 17 individuals, 0.25 mg - 17 individuals, and placebo - 20 individuals. Demographic characteristics of trial participants is presented in Table 1.

Table 1. Demographic characteristics of trial participants.

Group	Number of participants randomized	Number of men	Number of women	Average age
Placebo	20	9	11	33.6±6.2
0.25 mg	17	5	12	36.9±9.4
0.5 mg	17	9	8	37.1±8.5
Total	54	23	31	35.7±8.0

Vaccination was completed in 53 trial participants. One individual was prematurely withdrawn from the study due to a cold unlikely associated with vaccination.

Adverse events were registered in 17 out of 54 trial participants (31,8%). In vaccinated groups (0.25 mg and 0.5 mg combined) 35 AE in 12 patients were detected (35,3%), in placebo group - 13 AE in 5 patients (25,0%). In group receiving 0.25 mg of the vaccine AE were found twice more often than in group receiving dose 0.5 mg (47,1% and 23,5% respectively). Total data on adverse events registered in trial participants are presented in Table 2.

Table 2. Adverse events registered in trial participants.

Adverse event	DNA-4 0,25 mg		DNA-4 0,5 mg		DNA-4 0,25 mg + 0,5 mg		Placebo	
Number	17		17		34		20	
	N	%	N	%	N	%	N	%
fever	1	5,9	0	0,0	1	2,9	2	10,0
feeling of acid in the mouth	0	0,0	0	0,0	0	0,0	1	5,0
toothache	0	0,0	0	0,0	0	0,0	1	5,0
weakness	0	0,0	0	0,0	0	0,0	1	5,0
left arm pain	0	0,0	0	0,0	0	0,0	1	5,0
itching at the injection site	1	5,9	0	0,0	1	2,9	1	5,0
hyperemia at the injection site	1	5,9	0	0,0	1	2,9	0	0,0
menstrual disorders	2	11,8	0	0,0	2	5,9	0	0,0
hypersecretion from the genital tract	1	5,9	0	0,0	1	2,9	0	0,0
cold	3	17,6	3	17,6	6	17,6	0	0,0
food infection	1	5,9	0	0,0	1	2,9	0	0,0
high blood pressure	1	5,9%	0	0,0%	1	2,9%	0	0,0%
neutropenia	3	17,6%	1	5,9%	4	11,8%	1	5,0%
increased bilirubin	1	5,9%	0	0,0%	1	2,9%	1	5,0%
leukopenia	2	11,8%	2	11,8%	4	11,8%	1	5,0%

>50		0	2	1	3	0	0	0

SD - standard deviation; Min - minimum value; max - maximum value.

The relative frequency of the viral blips as well as the number of patients with viral blips in the placebo group and vaccinated groups did not differ. But the madnitude of some transient viral increases was much higher in groups receiving the candidate DNA vaccine. The biggest blips were detected in group 0.25 mg of the vaccine (patients # 21 and 37) and made up 2800 and 18000 copies/mL respectively. There were gradual increase and then decrease of viral load in participant #21.The third largest blip, 709 copies/mL, was found in 0.5 mg group in patient # 43.

3.3. CD4 and CD8 T cells measurement

The number of CD4 and CD8 T cells were measured by flow cytometry at visits 2, 6, 7, 9, 11. The results are shown in Tables 4 and 5.

Table 4. Data on CD4 T cells number in trial participants at different visits.

Group		Visit 2	Visit 6	Visit 7	Visit 9	Visit 11	T test
	N	16	17	15	15	13	
	Mean	0,593	0,619	0,722	0,703	0,756	
0.25 mg	SD	0,223	0,241	0,152	0,223	0,187	0,132
	Min	0,246	0,288	0,506	0,354	0,348	
	Max	1,114	1,290	1,176	1,121	1,056	
	N	17	17	14	14	11	
	Mean	0,769	0,714	0,710	0,671	0,797	
0.5 mg	SD	0,282	0,278	0,278	0,255	0,323	0,104
	Min	0,307	0,333	0,346	0,232	0,361	
	Max	1,281	1,267	1,275	1,093	1,196	
	N	20	20	17	16	12	
	Mean	0,558	0,620	0,603	0,555	0,650	
Placebo	SD	0,202	0,183	0,213	0,127	0,211	
	Min	0,189	0,353	0,278	0,239	0,367	
	Max	1,063	1,018	1,009	0,749	1,009	

Table 5. Data on CD8 T cells number in trial participants at different visits.

Group		Visit 2	Visit 6	Visit 7	Visit 9	Visit 11	T test
	N	16	17	15	15	13	
	Mean	0,939	0,937	1,059	1,014	0,937	0,306
0.25 mg	SD	0,540	0,450	0,533	0,534	0,293	
	Min	0,312	0,316	0,301	0,390	0,352	
	Max	2,376	1,926	2,199	2,230	1,406	
	N	17	17	14	14	11	
	Mean	1,037	0,992	0,902	0,847	1,033	0,969
0.5 mg	SD	0,479	0,380	0,276	0,390	0,451	
	Min	0,422	0,322	0,507	0,262	0,469	
	Max	2,020	1,870	1,287	1,635	2,033	
	N	20	20	17	16	12	
	Mean	0,976	0,976	0,940	0,923	1,086	
Placebo	SD	0,549	0,484	0,408	0,349	0,500	
	Min	0,285	0,332	0,364	0,437	0,476	
	Max	2,506	2,059	1,711	1,604	2,365	

There was a weak trend to increase in the absolute number of CD4 T cells in the group receiving 0.25 mg of the studied vaccine, but the differences with placebo group were not statistically significant.

4. Discussion

The phase II clinical trial was conducted as a multicenter, double-blind, placebo-controlled study of safety and dose selection of candidate HIV vaccine for HIV-infected people receiving ART.

The proportion of trial participants who demonstrated adverse events associated or possibly associated with ~~studied~~ vaccine administration was 7.4% higher in vaccinated group than in placebo group, that most likely due to ~~a~~ small size of groups. At the same time, the frequency of local reactions in group immunized with 0.25 mg of the vaccine and placebo group was similar, and in group immunized with 0.5 mg no local reactions were revealed.

There were no deaths or severe adverse events registered in the study. Significant differences in the dynamics of laboratory parameters, physical examination data, vital signs, and electrocardiograms between groups during the period of the clinical trial were not found.

It can be concluded that DNA-4 candidate vaccine at doses of 0.25 mg and 0.5 mg was safe and well- tolerated by HIV-infected individuals receiving ART.

For complete eradication of HIV infection the destruction of latent viral reservoirs is necessary, ~~that~~ cannot be achieved by modern ART. The only example of HIV cure is so-called "Berlin patient" who underwent allogeneic haematopoietic stem-cell transplantation (HSCT) from donor carrying homozygous mutation in the HIV coreceptor CCR5 [5, 6]. Recently ~~an~~ information about HIV-1 remission maintained over a further 18 months after ~~the~~ similar procedure was published [7]. ~~But~~ this procedure is very expensive, high-risky and cannot be widely used.

One of ~~of~~ approaches used for eliminating viral reservoirs is reactivation of latent proviral genomes during ART treatment by histone deacetylase inhibitors and some cytokines [8, 9].

Another way is enhancement of cellular immunity in HIV-infected individuals using therapeutic vaccines capable to induce functional $CD8^+$ T cells specific for HIV-1 epitopes [10-14]. The next generation of therapeutic vaccines will also be combined with reservoir activating agents [15]. DNA vaccine, in case of provirus activation, may be an ideal drug for viral reservoirs eradication.

Proviral genome reactivation may be caused by TNFα expression. TNFα activates transcription factor NFkB and HIV transciption [10, 16]. DNA-4 vaccination induced TNFα expression in some individuals, as ~~it~~ was shown in phase I clinical trial [3]. The expression of TNFα was also demonstrated by us in the cohort of exposed, seronegative individuals [4]. That is why we hypothesized that therapeutic "DNA-4" vaccine immunization may activate latent provirus and destroy at least some virus reservoirs.

In order to measure that, we assessed the frequency and magnitude of transient viral load increases above 50 copies/ml (blips). Such spontaneous viral load increases occurring during ART treatment are associated with latent viral reservoirs activation. There are several possible explanations of blips appearance including reactivation of latent HIV proviruses. The destruction of latent cells containing viral RNA can also be the source of the viral blips.

To investigate the effects of DNA vaccination on viral reservoirs we analyzed the magnitude and frequency of the blips in the placebo and immunized groups (Table 3). Neither relative frequency of the blips nor relative numbers of patients with blips differ between groups. But the amplitudes of blips in patients 21 and 37 immunized with 0.25 mg of the candidate DNA vaccine were much higher than that in placebo group - 2800 and 180000 copies/ml respectively. In participant #21 the increase of viral load was detected from 6^{th} to 10^{th} visit with the dynamics of increasing, peak and decreasing of viral load, while most of the other blips were detected only during single visit. The third largest blip, 709 copies / ml, was found in trial participant 43 vaccinated with 0.5 mg of DNA vaccine.

These results suggest that therapeutic DNA-4 vaccination during ART may cause the destruction of latent viral reservoirs in some patients due to reactivation of latent provirus.

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