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Safety & immunogenicity of tgAAC09, a recombinant adeno-associated virus type 2 HIV-1 subtype C vaccine in India

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Background & objective: A phase I trial of adeno-associated virus based HIV-1 subtype C vaccine (tgAAC09) was conducted at two sites in Germany and Belgium and one site in India. This paper reports the safety and immunogenicity of tgAAC09 in healthy adult Indian volunteers.

Methods: Between January 2005 and December 2006, 30 consenting volunteers were enrolled in the placebo controlled double-blind dose-escalation trial [3×10^9 , 3×10^{10} and 3×10^{11} DNase resistant particles (DRPs)/ml]. Single injection of the candidate vaccine was administered to ten volunteers randomized in 8:2 ratio in vaccine and placebo arms at each dosage level.

Results: The mean age of study volunteers (16 men and 14 women) was 34 yr. Six local reactogenicity events and 14 systemic reactogenicity events like malaise, fever, headache and myalgia were reported, both were dose-dependent. The difference between the adverse events reported by vaccine and placebo recipients (79 and 67%) was not significant. A modest IFN- γ ELISPOT response [248 spot forming units (SFU)/million cells] was detected in one volunteer from high dose group and low response (56 and 75 SFU/million cells) in two volunteers in low and mid-dose groups. A post-vaccination dose-dependent increase was observed in anti AAV2 neutralizing titres. None of the volunteers showed a positive antibody response to HIV-1.

Interpretation & conclusions: The trial was a benchmark in phase I clinical evaluation of HIV candidate vaccines in India. The vaccine was generally well tolerated and raised no safety concerns. The vaccine was found to be weakly immunogenic. It is essential to understand the role of pre-existing immunity against vectors and significance of evaluation in a prime-boost strategy.

Key words Adeno-associated - clinical trial - HIV candidate vaccine - Phase I - safety - tgAAC09

The Joint United Nations Program for HIV/AIDS has recently reported that the number of people living with HIV worldwide continued to grow in

2008, reaching an estimated 33.4 million (31.1 - 35.8 million). The total number of people living with the virus in 2008 was more than 20 per cent higher than

the number in 2000, and the prevalence was roughly three-fold higher than in 1990¹. Over 80 per cent of the new HIV infections are believed to be occurring in Sub-Saharan Africa and South and South East Asia.

The HIV epidemic has spread to all parts of India in the past two decades following the first report of HIV infection in Chennai in 1986. According to National AIDS Control Organization (NACO) of India, approximately 2.31 million people were living with HIV as of end of 2007 and the current adult HIV prevalence in India is estimated to be 0.34 per cent². The epidemic has been spreading from high risk to low risk populations and from urban to rural areas³⁻⁶. Classical prevention approaches like ABC of prevention (abstinence, behavioural change and condom use) have demonstrated success only in a few intervention studies and there are some limitations to acceptance of these prevention strategies⁷.

Introduction of highly active anti-retroviral therapy (HAART) has demonstrated major success in reducing the HIV associated morbidity and mortality, and improving the quality of life; however, even today, more than 90 per cent of the eligible patients are not able to receive anti-HIV drugs. Anti-retroviral therapy (ART) is a lifelong therapy, expensive and accessible to only a few and has additional limitations due to side-effects and problems in drug adherence resulting in drug resistance⁸. Hence in a resource-limited setting like India, prevention oriented options need to be critically viewed. Female oriented HIV prevention options like vaginal microbicides have faced major setbacks in the past few years⁹ and female condoms are still not widely accepted and available¹⁰. Newer prevention technologies like pre- and post-exposure prophylaxis are still under clinical evaluation¹¹. Considering the success of vaccines in preventing infectious diseases, a preventive vaccine against HIV seems to be the logical choice^{12,13}.

Over a hundred different vaccine candidates have been tested in human volunteers in different phases of clinical trials all over the world. Currently more than 30 vaccine trials are ongoing globally¹⁴. Majority of the HIV vaccine candidates are based on approaches like vector based vaccines, DNA vaccines or subunit vaccines. Modified vaccinia ankara (MVA), canarypox, adenovirus and adeno-associated virus (AAV) are among the commonly used vectors in HIV vaccines currently under clinical development. In a highly populous country with noticeable presence of HIV and a large susceptible population, the decision to support

vaccine development and field testing and evaluate a vaccine candidate based on the most commonly prevalent HIV-1 subtype C was natural.

A multi-centric multi-country phase 1 trial to assess safety and immunogenicity of HIV-1 subtype C based AAV vaccine (tgAAC09) was conducted at two sites in Germany, two sites in Belgium and one site at the National AIDS Research Institute (NARI), Pune, India. During this trial NARI enrolled 30 healthy volunteers¹⁵. We report here the detailed country specific findings on safety and immunogenicity of the candidate vaccine tgAAC09 at Pune, India. Being the first phase 1 trial of any vaccine in India, various processes employed in creating the infrastructure, building the capacity and implementing the trial are also discussed.

Material & Methods

Study approvals: Under the tripartite agreement signed by the National AIDS Control Organization (representing Ministry of Health and Family Welfare), Indian Council of Medical Research (ICMR) and International AIDS Vaccine Initiative (IAVI), a proposal for India to participate in a phase 1 trial of adeno-associated virus based HIV-1 subtype C vaccine (tgAAC09) was developed. The project proposal was evaluated and approved by the National Advisory Board on HIV vaccine. The trial was also approved by the Institutional Ethics Committee, the Drugs Controller General of India (DCGI), the Genetic Engineering Approval Committee (GEAC) of Ministry of Environment and Forests, the Health Ministry Screening Committee (HMSC), Government of India, the Scientific Advisory Sub-committee constituted by the ICMR, and the Central Ethics Committee of ICMR (clinical trials registry number: NCT00482027).

Study team training: The investigators visited ongoing HIV vaccine trial sites in Thailand and received specialized training in recruitment procedures, human subjects' research, good clinical and laboratory practices and gender related issues. A special Vaccine Trial Center was set up in NARI to conduct this trial.

Study design: The study vaccine (tgAAC09) consisted of purified particles of a recombinant adeno-associated virus serotype 2 (rAAV2) protein capsid containing single stranded DNA encoding HIV-1 subtype C gag-PR- Δ RT genes (South African isolate, DU422). The study vaccine tgAAC09 was formulated in a sterile isotonic buffered salt solution, which also served as placebo¹⁵. In this phase-1 dose-escalation trial, three dosages of the candidate vaccine [3 x 10⁹, 3 x 10¹⁰ and

3×10^{11} DNase resistant particles (DRPs) per ml] were evaluated in three groups of ten volunteers each. Of the ten volunteers in each group, 8 volunteers received the vaccine and two received the placebo through random assignment. Two weeks following the last enrollment in the low dose group, an external Safety Review Board constituted by the study sponsors evaluated the safety data and allowed the investigators to start enrollment in the mid-dose group. Similar procedures were followed while moving from the mid to the high dose group. Typically, for any study volunteer, the visit schedule consisted of a screening visit, enrollment visit and 10 follow up visits in addition to two telephonic contacts in the first week after enrollment.

Informed consent process and other ethical issues: The study volunteers were given assurance that information and details about their study participation will be kept confidential and will not be revealed to unauthorised persons. The investigators and an expert group of 20 members from India developed screening and enrollment consent forms and a test of comprehension for this study. In two-step consent process the participants signed the screening consent form first at the screening visit and eligible and willing participants returning for enrollment visit were administered the enrollment informed consent. The volunteers had to pass a test of comprehension of the enrollment consent document following which they signed the enrollment consent forms. The participants were informed that they would be entitled to receive complete treatment for any condition related to study product administration. They were given health insurance for the duration of the study. They were assured that they could contact the institute in case of any problem even in the future and appropriate referral and care support would be offered to them.

Screening and randomization: The study was conducted between January 2005 and December 2006 among healthy consenting volunteers in the age group of 18 to 49 yr and at low risk of HIV infection who were identified through community sensitization. Individuals with current or past chronic medical conditions, abnormal laboratory values, history of receiving blood products or vaccines in the recent past and history of allergic reactions to vaccines were excluded. Pregnant or lactating women and those planning pregnancy within 4 months after receiving the vaccine were also excluded. Following screening for eligibility determination, volunteers were asked to return for enrollment within 6 wk.

Enrollment, randomization and follow ups: The enrollment in the trial was initiated in January 2005 and the trial was completed in December 2006. At the enrollment visit the volunteers were randomized into vaccine and placebo arms in a ratio of 8:2 at each dosage level. The investigators and study volunteers were blinded to the type of study product that was administered at the enrollment visit after signing the enrollment consent. There was no blinding at the dosage group level. Enrolled volunteers received a single injection of 0.5 ml of the study product (tgAAC09 or placebo) intramuscularly in the deltoid muscle of the non-dominant arm. Counselling was provided at every clinic visit which included pre- and post-HIV test counselling, contraception counselling, HIV risk reduction counselling and counselling to ensure adherence to study procedures. Study participants were evaluated during 10 clinic visits spread over 12 months of follow up following the study product administration. Blood samples were collected at screening, enrollment and at weeks 2, 4, 12, 24, 36, and 52 for safety and immunogenicity assessments.

Safety and tolerability assessment: Vital signs were recorded and the volunteer was observed for any untoward reaction for four hours after the study injection. Local and systemic reactogenicity events were recorded among study participants within 7 days of study product administration. The study participants were advised to immediately contact the study team or visit the study clinic for any medical or non-medical problems. Adverse events (AEs) including laboratory abnormalities were recorded up to 24 wk and serious adverse events (SAEs) were recorded during the entire trial period of one year following the study injection. The severity of AEs was graded using the DAIDS (Division of AIDS), National Institute of Allergy and Infectious Diseases (NIAID) toxicity grading table [www.niaid.nih.gov] and the relationship to the study product was assessed and categorized as unrelated, possibly related, probably related or definitely related to the investigational product.

Assessment of immunogenicity: Volunteers were tested for HIV-1 and HIV-2 antibodies by ELISA using a commercial diagnostic kit (Bio-Rad Laboratories ELAVIA Ac-Ab-Ak 1, USA) that uses whole virus as antigen at weeks 0, 4, 12, 24, 36 and 52. Cellular immunogenicity was assessed using IFN- γ secretory ELISPOT assay. The ELISPOT assays were performed on fresh peripheral blood mononuclear cells (PBMCs) at NARI, Pune and on frozen cells at IAVI Human

Immunology Laboratory (HIL), London, UK. The number of spot forming units (SFU) per 10^6 PBMC was counted using an automated ELISPOT reader (AID, Germany). Responses were considered positive (1) if the SFUs in the peptide wells were greater than 38-60 SFU/ 10^6 cells (depending upon the peptide pool) above the background and were more than four times the mean background SFU count, and (2) if the SFU in the peptide wells demonstrated less than 70 per cent coefficient of variation across the replicate wells and had a background of <55. Impact of pre-existing immunity to AAV2 on vaccine-induced responses was measured by serum anti-AAV2 capsid neutralizing titres using a microtitre assay of Targeted Genetics Corporation, USA.

Statistical analysis: The safety and immunogenicity outcomes were compared among vaccine recipients in the three dosage groups (8 in each group) and between each group and placebo recipients (6 volunteers). The proportion of volunteers with HIV-specific ELISPOT responses and differences between volunteers who developed a four-fold or greater rise in serum neutralizing antibodies to AAV2 after vaccination and those who did not, were compared.

Results

Profile of participants: A total of 80 persons voluntarily participated in 99 screening evaluations and 40 volunteers were found to be eligible to participate in the vaccine trial (Table I). Of these, 30 were enrolled of whom 16 were men and 14 were women. The retention of participants in the study was 100 per cent with all the study participants completing the stipulated study visits.

The mean age of study participants was 34 yr (range 20-49 yr). The mean age and the age distribution of the male and female participants were similar. More men had completed higher level of education compared to female participants (75% versus 43%). More than 50 per cent men were in salaried class; while 3 each were either self-employed or factory workers. Majority of the women (11/14) were either grass-root level workers in the community or social workers. One male participant and 2 female participants were health-care providers. Five men and one woman were unmarried and the remaining volunteers were married.

Safety: Overall, 6 different local reactogenicity events were reported during the first seven days after the investigational product administration by 4 volunteers (1 placebo recipient, 2 vaccine recipients in the high

Table I. Socio demographic profile of Phase-1 HIV vaccine trial participants of tgAAC09 in India

Category	Sub-categories	Male (n= 16)	Female (n= 14)
Age (yr)	Up to 30	08	06
	31- 40	04	04
	41- 50	04	04
Education	Up to 10 th	04	08
	College education	08	05
	Graduation & above	04	01
Occupation	Community/ social worker	-	11
	Factory worker	03	00
	Self-employed	03	00
	Salaried	09	01
	Health-care providers	01	02
Marital status	Married	11	13
	Unmarried	05	01

dose group and 1 in the mid-dose group)¹⁵. The local reactogenicity was found to be dose-dependent. Mild to moderate pain at injection site was the most commonly reported local reactogenicity event. One volunteer in the mid-dose group experienced induration and tenderness in addition to pain at the injection site. One local reactogenicity event was treated with an analgesic, while the rest did not require any medication.

Malaise, fever, headache and myalgia were the most commonly reported systemic symptoms¹⁵. In all, 14 systemic reactogenicity events were reported by 5 vaccine [3 in the low dose group and 1 each in the mid and high dose groups] and 1 placebo recipients (Table II) and only one volunteer required medication. Data might suggest a dose- response trend, but only 3 out of 14 systemic events were severe in nature and since all of them had some underlying cause, they were judged as 'not related' to the investigational product.

The adverse events (AEs) recorded during 24 wk after the study product injections have been summarized in Table II. Overall, 19/ 24 (79%) vaccine recipients and 4/6 (66.7%) placebo recipients reported 45 adverse events (37 and 8 among vaccine and placebo recipients respectively) and this difference was not statistically significant. Of these, 19, 8 and 10 events were reported by study volunteers in low, mid and high dosage groups and were not related to study product administration. Adverse events were noted among 69 per cent male and 86 per cent female volunteers. The differences in adverse events reported by recipients of different dosage groups and between vaccine and placebo groups were not statistically significant. Of the total 45 events, 23

(51%) were mild, 15 (34%) were moderate, six (13%) were severe and one was graded as very severe. They were evenly distributed across the three vaccine dose groups. A majority [35/45, 78%] of the AEs required treatment or medication. Three AEs (7%) were judged as possibly related to the study product and no AE was considered to be probably or definitely related to the study vaccine. Musculoskeletal symptoms (38%) and respiratory system related ailments (24%) were the commonest AEs reported (data not shown). Other reported AEs included abnormal laboratory values (7%) and traumatic injuries (7%). However, none of the three volunteers with laboratory abnormalities had associated symptoms and the laboratory values returned to normal on repeat evaluations.

There were two serious adverse events [SAEs] resulting from hospitalization of two participants. One volunteer in the low dosage group was hospitalized for 12 days from day 199 after enrollment for chest pain and breathlessness following a family dispute and was diagnosed to be suffering from "acute panic disorder". Another volunteer in the mid dose group had severe pain in the right lower limb (sciatica) from day 189

after enrollment. He was diagnosed to have nerve sensitization due to inter-vertebral disc protrusion (L4-L5). Spinal nerve root block procedure was performed twice which resulted in recovery approximately 2 wk following the end of the study participation at week 52. In view of the identifiable possible aetiological condition and lack of temporal relationship, both the SAEs were judged as unrelated to the study product administration.

Cellular immunogenicity: The IFN- γ ELISPOT response was detected using both frozen as well as freshly isolated peripheral blood mononuclear cells only in 1 of 8 Indian volunteers who received the high dose (3×10^{11} DRP)¹⁵. The response was modest (248 SFU/million cells) and detected at wk 24. It persisted during subsequent visits at week 36 (73 SFU/million cells) and wk 52 (59 SFU/million cells) although was lower in magnitude. Two additional volunteers, one each from the low dose (56 SFU/million cells at wk 24) and mid dose group (75 SFU/million cells at wk 52), were detected as responders using frozen PBMCs, however the responses were closer to the limit of detection.

Table II. Local reactogenicity, systemic reactogenicity and adverse reactions among Indian vaccine trial participants receiving tgAAC09 or placebo

Safety parameters	Placebo recipients (n = 6)	Vaccine recipients (n = 24)	Low dose recipients (n = 8)	Mid dose recipients (n = 8)	High dose recipients (n = 8)
<i>Adverse events:</i>					
No. of adverse events (AEs)	8	37	19	8	10
<i>Severity</i>					
Mild	5	18	8	3	7
Moderate	2	13	8	4	1
Severe	1	5	3	1	1
Very severe	0	1	0	0	1
<i>Relationship</i>					
Not related	4	11	6	4	1
Unlikely	4	23	11	4	8
Possibly	0	3	2	0	1
Probably	0	0	0	0	0
Definitely	0	0	0	0	0
<i>Medication required</i>					
Yes	5	30	14	8	8
No	3	7	5	0	2
<i>Visit type</i>					
Scheduled	6	22	12	3	7
Unscheduled	2	15	7	5	3
Volunteers with AEs	4	19	7	5	7
<i>Serious adverse events:</i>	0	2	1	1	0

Anti-AAV2 capsid neutralizing titres and anti-HIV antibodies: The baseline AAV2 neutralizing antibody titres were high in the Indian volunteers [29 of 30 volunteers (97%)] with a geometric mean titre of 1/75 and titre ranging from 1/4 to 1/4096 (data not shown). A dose-dependent increase (four-fold or greater) was observed after vaccination with tgAAC09. The proportion of volunteers showing a four-fold increase in titres was 1/6 in placebo group, 2/8 in the low dose, 3/8 in the mid dose and 8/8 in the high dose. No anti-gag antibodies were detected in the serum samples of any of the volunteers at any of the study visits.

Discussion

Considering the magnitude of HIV epidemic in India, it is important to evaluate emerging HIV prevention options such as HIV vaccines and vaginal microbicides. We evaluated in a phase I study a vaccine construct tgAAC09 based on HIV-1 subtype C, which is the main subtype circulating in India¹⁶. Currently vector-based vaccines, subunit vaccines, and DNA vaccines are the commonly used vaccine candidates in human trials¹⁷. Proven safety of the vector in preclinical as well as in various human gene therapy trials, robust immunogenic response observed in the preclinical study and simplicity of administration made the AAV-based HIV-1 subtype C vaccine a potential candidate for evaluation among Indian volunteers.

Being India's first phase I HIV vaccine trial, getting support from policy makers, programme managers, health care providers and the community at large were the initial goals and they were adequately achieved. Trial conduct helped set a benchmark in clinical evaluation of HIV candidate vaccines in phase I trials in India. The vaccine trial center at NARI in Pune enrolled 30 volunteers within 11 months after trial initiation. The dose-escalation from low dosage group to high dosage groups was closely monitored and approved by an external independent Safety Review Board. Both genders were almost equally represented among volunteers and their socio-economic profile reflected that of the general population. All the follow up evaluations among the vaccine trial participants were completed with 100 per cent retention in this trial.

During the follow up observations, no major safety concerns were noted and the vaccine candidate was generally well-tolerated at all dosage levels. Although reactogenicity showed some indication of a dose-response relationship, the differences observed

between the vaccine recipients in the three dosage-groups and between the vaccine recipients and the placebo recipients were not statistically significant. About 76 per cent of the 30 volunteers reported some adverse event, but there was no dose response relationship in reporting of adverse events among the three groups of volunteers. The local and systemic reactogenicity events observed in this Phase I study were comparable with those observed in other Phase I trials^{18,19}. In a sensitive trial like that on HIV vaccine, it is expected that participants might report even minor symptoms, which they would have otherwise ignored in normal circumstances. This may explain why a majority of the AEs were reported during scheduled visits of volunteers to the clinic. Only 17 events (38%) were self-reported on an unscheduled visit. The two SAEs reported at the site were judged to be unrelated to the study vaccine because of lack of temporal relationship and other evident causative etiologies. Overall safety of the vaccine candidate tgAAC09 was consistent with various phase I trials worldwide²⁰⁻²². Considering the data on reactogenicity and AEs, this vaccine (tgAAC09) appears to be safe for human use at all tested dosage levels during the study period of one year. The safety of even higher dose (3×10^{12} DRP) of this product has been demonstrated recently in a phase II trial in 91 participants from South Africa, Zambia and Uganda (unpublished data of International AIDS Vaccine Initiative).

The vaccine was not able to induce a strong immune response among Indian volunteers despite a very good immunogenicity profile in preclinical studies. Similar findings were also reported from the tgAAC09 vaccine trial participants from Germany and Belgium¹⁵. Only one of eight Indian volunteers receiving single injection of high dose showed a positive immune response. Similar weak immunogenicity response was also reported in African trial of the same vaccine candidate with 12 per cent volunteers showing HIV-specific T-cell responses after first vaccination and 19 per cent after subsequent vaccination given 6 months apart²³. Preclinical studies had shown that vaccine DNA packaged in AAV capsids is immunogenic after intramuscular injections. In mice and macaques robust and persistent responses were observed²⁴. However, for unclear reasons, animal findings did not translate into comparable responses in humans.

It appears that AAV-2 sub-clinical infections are fairly common in India as a significant proportion of volunteers (including 1 out of 6 in placebo group)

showed a four-fold rise in the AAV-2 neutralizing antibody titres. The baseline prevalence of AAV2 capsid neutralizing antibodies was much higher in Indian trial participants (97%) compared to their European counterparts (48%)¹⁵. The data from all volunteers in the multi-country study indicated that prior to first vaccination, the baseline anti-AAV2 capsid neutralizing titres for the 6 vaccine recipients who had IFN- γ ELISPOT responses post first vaccination ranged from 4 to 409,615. Antibody response was observed to be dose-dependent which was further enhanced with booster vaccination with tgAAC09. One responder in IFN γ - ELISPOT assay also had high baseline anti-AAV2 capsid neutralizing antibody titre (1 in 128). Hence, it is not possible to comment on the impact of pre-existing immunity against AAV-2 on the generation of antigen-specific immune response and needs further exploration. The reasons for differences in the estimated IFN- γ responses shown between freshly isolated and frozen lymphocytes remain unclear.

In spite of the candidate vaccine's failure to generate the desirable immune response, this trial has proved that world-class HIV vaccine trials can be successfully conducted in India. The trial team worked closely with the community, stakeholders, policy makers and programme managers to seek support at various levels. The trial was adequately monitored. The trial team succeeded in enrolling appropriate volunteers and completing the trial without any dropouts. Presumably this trial will pave the way for more speedy conduct of trials in India in future and can be considered as a demonstration of capacity building and preparedness of the trial team to undertake and successfully complete a sensitive trial of HIV vaccine in India. Although unlikely to proceed further in clinical development as a single component, tgAAC09 might deserve to be evaluated in prime-boost strategies.

The recent failures of the Merck Ad5 STEP and Phambili trials resulted in questions being asked on relevance of the current vaccine approaches in both the scientific community and the general public²⁵. However, it is critical to continue well-designed and fully justified efficacy trials with other emerging vaccine candidates using other vaccination approaches as HIV vaccine still remains the best hope for prevention of HIV infection²⁶⁻²⁹. The recently declared Thailand trial results demonstrating 31 per cent protective efficacy of a prime boost combination of two vaccines can be considered as a ray of hope in this direction³⁰. In India, the focus should be on development and testing of

vaccine candidates that are deemed most relevant in the context of epidemiological findings related to the viral and host factors. The more the vaccine candidates are evaluated; the better will be the chances of finding a suitable candidate for the country.

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