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Safety and Preliminary Efficacy of CS-based Recombinant Adenoviral Serotype 35 and Serotype 26 Malaria Vaccine Candidates in Prime-Boost Vaccination of Healthy Adults Undergoing Subsequent Experimental *P. falciparum* Sporozoite Challenge

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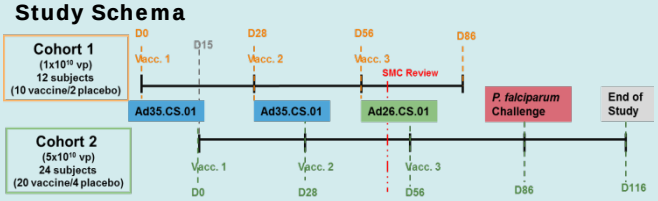
Introduction

Development of an effective vaccine remains a major challenge in combating malaria. While pre-erythrocytic vaccines eliciting high levels of antibody against circumsporozoite (CS) antigen have been shown to provide protection against malaria infection, pre-clinical studies suggest a role for cellular immune responses in protection against malaria infection. This was evaluated in a phase 1/2a study assessing the safety, tolerability and immunogenicity of the prime/boost administration of the Ad35.CS.01/Ad26.CS.01 candidate malaria vaccines to healthy adult subjects at two dose levels, followed by an evaluation of the protective efficacy of the higher dose level in an experimental malaria challenge. This was a first-in-human administration of the Ad26.CS.01 vaccine.

Study Schema



Materials and Methods

Vaccines: The Ad35.CS.01 and Ad26.CS.01 vaccines are recombinant human adenovirus serotype 35 and serotype 26 vectors carrying the gene encoding the *P. falciparum* circumsporozoite (CS) surface antigen. Normal saline was used for placebo.

Study Population: A total of 42 healthy malaria-naïve male and non-pregnant female subjects aged ≥18 to ≤50 years were enrolled. Thirty six subjects in two cohorts were enrolled in the Vaccination Phase. Cohort 2 vaccine subjects and an six (6) unvaccinated infectivity controls were enrolled in the Challenge Phase.

Vaccination: Subjects in the low-dose (1x10¹⁰ vp) and high-dose (5x10¹⁰ vp) cohorts each received two vaccinations with Ad35.CS.01/placebo followed by vaccination with Ad26.CS.01/placebo at monthly intervals (Figure 1). Vaccinations between cohorts were staggered by 15 days to allow for adequate review of the safety data. Solicited local and systemic adverse events (AEs) were collected for seven days following vaccination. Unsolicited AEs were collected throughout the study. All subjects were followed for 6 months after the last vaccination and/or challenge for occurrence of serious adverse events (SAEs).

***P. falciparum* challenge**: Four weeks after the last vaccination, Cohort 2 vaccine subjects and infectivity controls underwent experimental infection with *P. falciparum* sporozoites by the bites of five infected *A. stephensi* mosquitoes. Subjects were closely monitored for signs and symptoms of malaria and from day 9-18 post-challenge, had daily peripheral blood smears to assess for development of patent (microscopic) parasitemia. All subjects were treated and cured of their malaria infection immediately upon diagnosis or at the end of the study if they remained blood smear negative.

Immunology Assays: Anti-CS antibodies, neutralizing antibodies to Ad26 and Ad35, and CD4 and CD8 T-cell responses against CSP were evaluated at baseline and D28 following each vaccination and malaria challenge. CS ELISA was validated and performed as previously described.[1] Ad35 and Ad26-specific neutralizing antibody titers were assessed by validated luciferase-based virus neutralization assays as previously described.[2] Cellular responses were evaluated at baseline, D14 and D28 following each vaccination and malaria challenge using cryopreserved peripheral blood mononuclear cells (PBMC) stimulated with 59 CS peptides (2 µg/ml/peptide), consisting of overlapping 15-mer peptides, representing the full-length CS protein. For the ELISPOT assay, precoated human α-IFNγ ELISPOT plates incubated overnight were washed and spots visualized with detection antibody 7-B6-1-ALP and BCIP/NBT. Spots were quantified by digitally imaged automated spot counting. For Intracellular Cytokine Staining (ICS), Golgi Plug was included during the 6-hrs after which staining was performed with aqua fluorescent Dye A , APC H7-α-CD3, Horizon V450-α-CD4 and PerCP-Cy5.5-α-CD8, APC-α-IFNγ, FITC-α-TNFα and PE-α-IL-2. Data was acquired and analyzed by FACSCanto II flowcytometer and FACSDiva 6.1.2 software.

Results

General: No SAEs were reported during the study period and all subjects completed the study. All Cohort 1 and Cohort 2 subjects received both Ad35.CS.01 vaccinations. All Cohort 1 (10/10) and 18/20 Cohort 2 subjects received the Ad26.CS.01 vaccine. A total of 23 subjects underwent *P. falciparum* challenge, including 17/20 Cohort 2 vaccine subjects and 6 infectivity controls.

Vaccination Phase: Similar reactogenicity profiles were seen in the low-dose vaccine and placebo subjects, with the incidence of AEs slightly higher in the high-dose vaccine cohort. Incidence of injection site pain was similar in both vaccine and the placebo subjects whereas injection site erythema, induration and swelling were reported in the high dose group (one report of erythema, two each of induration and swelling). The incidence of solicited local AEs was similar after each vaccination. All solicited local reactions were mild to moderate in severity and resolved without sequelae. Two subjects reported solicited transient neurological events (paraesthesia), one from the low dose group and one from the high dose group.

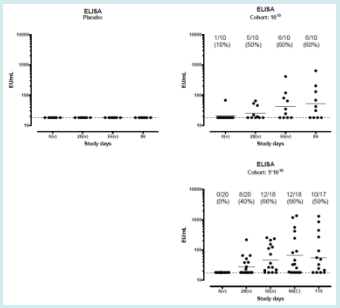
The most commonly reported solicited systemic AEs after each vaccination were malaise, headache, and myalgia. The incidence of these AEs was higher after the third vaccination (Ad26) than after the first or second vaccinations (Ad35). Six subjects (16.7%) reported severe vaccine-related AEs, including one placebo subject (dizziness) and five high dose vaccine subjects (two reports of pyrexia, three of headache, two of malaise, one of myalgia and two of chills).

All subjects reported unsolicited AEs and 24 subjects (66.7%) reported unsolicited AEs related to study vaccine. In general, reporting of unsolicited AEs was higher in the high dose group. The most commonly reported unsolicited AE after each dose was upper respiratory tract infection. Vaccine-related unsolicited AEs reported by more than one subject after the first dose were hyperhydrosis, increased blood glucose, dizziness and proteinuria; after the second dose: anemia; and after the third dose: hyperhidrosis.

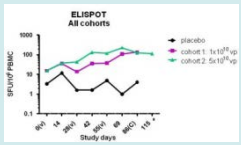
Challenge Phase: The mean incubation period was 9.22 days (range 5-12 days). Approximately 50% of subjects were asymptomatic until the day of diagnosis. The most common related AEs were headache (30/320; 9.4%), application site pruritis (23/320; 7.2%), back pain (21/320; 6.6%), myalgia (21/320; 6.6%), chills (20/320; 6.3%) and arthralgia (16/320; 5.0%). The majority of these events were reported as mild in severity. The only severe related AEs were chills (1/20; 5.0%), leukopenia (1/7; 14.3%), pyrexia (2/8; 25.0%) and thrombocytopenia (2/10; 20.0%). All subjects reported AEs during the Challenge Phase.

Infectivity: Of 23 subjects experimentally infected, 6/6 infectivity controls and 16/17 Cohort 2 vaccine subjects developed patent parasitemia (malaria diagnosis). One vaccinated subject was protected, remaining blood smear negative throughout the Challenge Phase. The mean pre-patent period for vaccinees was 10.18 days (range 9-13 days) and for infectivity controls was 10.33 days (range 9-12 days). The duration of parasitemia following diagnosis and treatment ranged from one to three days (mean 1.32 days).

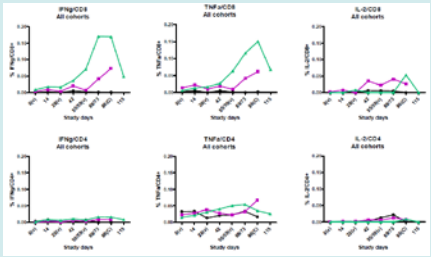
Immunology



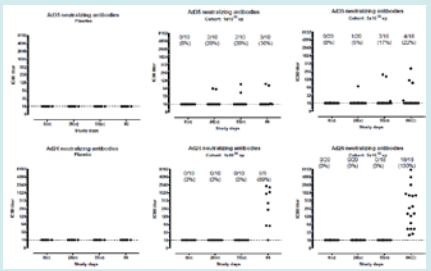
- CS Abs are elicited in around half of the subjects
- GMTs indicate a dose response



- A clear increase in ELISPOT responses can be seen after vaccination

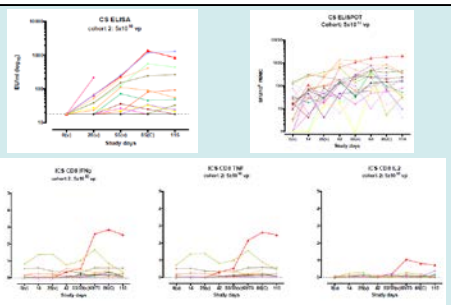


- ICS detects mainly CD8 responses with very little CD4 responses
- CD8 TNFα and IFNγ responses after vaccination are markedly increased compared to placebo and boosted upon administration of the Ad26.CS.01 boost



- While Ad35 elicits only few and modest NAb titers, Ad26 elicits NAbs in most subjects with high levels after just one vaccination

Protected subject shows highest CS antibody titers and T cell response at day of challenge



Conclusions

In general, both the Ad35.CS.01 and the Ad26.CS.01 vaccines were well tolerated at the 1x10¹⁰ vp and 5x10¹⁰ vp dose levels. This is the first-in-man study of the Ad26.CS.01 candidate malaria vaccine.

- **Vaccine reactogencity profile was similar between low dose and placebo groups, with slightly higher systemic reactogenicity observed at the higher dose level. Symptoms were more frequent following the third vaccination (Ad26.CS.01).**
- **One subject out of 17 demonstrated sterile protection against *P. falciparum*. This subject also showed highest CS antibody titers and T cell response at the time of malaria challenge.**
- **Up to 67% of subjects developed CS ELISA titers after vaccination**
- **Ad35.CS.01 vaccination elicited modest neutralizing adenoviral vector antibodies, while Ad26.CS.01 vaccination elicited neutralizing anti-vector antibodies in almost all subjects, at higher levels**
- **By ELISPOT, T cell responses are consistently higher in vaccine groups compared to placebo**
- **ICS detects mainly CD8 T cell responses producing TNFα and IFNγ with very little CD4 response**
- **Experimental human malaria infection model is safe and enables critical early vaccine development decisions.**

[1]Kostense et al. Clin Vaccine Immunol 2011
[2]Sprangers et al. J Clin Microbiol 2003

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