

Protection Against Malaria by Intravenous Immunization with a Nonreplicating Sporozoite Vaccine

Robert A. Seder,^{1*} Lee-Jah Chang,^{1*} Mary E. Enama,¹ Kathryn L. Zephir,¹ Uzma N. Sarwar,¹ Ingelise J. Gordon,¹ LaSonji A. Holman,¹ Eric R. James,² Peter F. Billingsley,² Anusha Gunasekera,² Adam Richman,² Sumana Chakravarty,² Anita Manoj,² Soundarapandian Velmurugan,² MingLin Li,³ Adam J. Ruben,² Tao Li,² Abraham G. Eappen,² Richard E. Stafford,^{2,3} Sarah H. Plummer,¹ Cynthia S. Hendel,¹ Laura Novik,¹ Pamela J.M. Costner,¹ Floreliz H. Mendoza,¹ Jamie G. Saunders,¹ Martha C. Nason,⁴ Jason H. Richardson,⁵ Jittawadee Murphy,⁵ Silas A. Davidson,⁵ Thomas L. Richie,⁶ Martha Sedegah,⁶ Awalludin SutamiHardja,⁶ Gary A. Fahle,⁷ Kirsten E. Lyke,⁸ Matthew B. Laurens,^{8,9} Mario Roederer,¹ Kavita Tewari,¹ Judith E. Epstein,⁶ B. Kim Lee Sim,^{2,3} Julie E. Ledgerwood,¹ Barney S. Graham,¹ ‡ and Stephen L. Hoffman,^{2,3} ‡ the VRC 312 Study Team§

¹Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20852, USA. ²Sanaria, Rockville, MD 20850, USA. ³Protein Potential LLC, Rockville, MD 20850, USA. ⁴Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20852, USA. ⁵Entomology Branch, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA. ⁶U.S. Military Malaria Vaccine Program, Naval Medical Research Center, Silver Spring, MD 20910, USA. ⁷Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD 20892, USA. ⁸Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD 21201, USA. ⁹Howard Hughes Medical Institute, Baltimore, MD 21201, USA.

*These authors contributed equally to this work.

†To whom correspondence should be sent. E-mail: rseder@mail.nih.gov

‡These authors contributed equally to this work.

§The VRC 312 Study Team members are listed in the supplementary materials.

Consistent high-level, vaccine-induced protection against human malaria has only been achieved by inoculation of *Plasmodium falciparum* (Pf) sporozoites (SPZ) by mosquito bites. We report that the PfSPZ Vaccine, composed of attenuated, aseptic, purified, cryopreserved PfSPZ, was safe and well-tolerated when administered 4-6 times intravenously (IV) to 40 adults. 0/6 subjects receiving 5 doses, 3/9 subjects receiving 4 doses of 1.35×10^5 PfSPZ Vaccine, and 5/6 non-vaccinated controls developed malaria following controlled human malaria infection ($P = 0.015$ in the 5-dose group and $P = 0.028$ for overall, both versus controls). PfSPZ-specific antibody and T cell responses were dose-dependent. These data indicate there is a dose-dependent immunological threshold for establishing high-level protection against malaria that can be achieved by IV administration of a vaccine that is safe and meets regulatory standards.

Malaria control interventions, including insecticide-impregnated bednets, insecticide spraying, and antimalarial drugs, have reduced malaria morbidity and mortality substantially (1). However, in 2010 despite these measures, there were an estimated 220 million clinical cases and 0.66 to 1.24 million deaths caused by malaria (1, 2). A highly effective vaccine will be ideal for preventing malaria in individuals and eliminating malaria in defined geographic areas. It would optimally target the parasite at asymptomatic, pre-erythrocytic stages (3, 4). The World Health Organization malaria vaccine technology roadmap set a vaccine efficacy goal of 80% by 2025 (5). Heretofore, no injectable malaria vaccine candidate has consistently approached that level of efficacy.

RTS,S/AS01 is the most advanced subunit malaria vaccine, protecting approximately 50% of subjects against controlled human malaria infection (CHMI) 2-3 weeks after the last dose of vaccine and 22% at 5 months after last immunization (6). In a large-scale phase 3 efficacy trial in African infants 6-12 weeks of age, RTS,S/AS01 reduced the rates of clinical and severe malaria acquired over a 12 month period by 31.3% and 36.6%, respectively (7).

It has been known for 40 years that it is possible to achieve high-level, sustained, protective immunity against the pre-erythrocytic stages of the parasite through immunization by the bites of $>1,000$ irradiated mosquitoes carrying *Plasmodium falciparum* (Pf) sporozoites (SPZ) (8-11). Advancing this technique beyond administration by mosquitoes required the capacity to manufacture aseptic, radiation attenuated, metabolically active, purified, cryopreserved PfSPZ for an injectable vaccine that met regulatory standards (12). This was achieved (13, 14) and the first clinical trial of the PfSPZ Vaccine, composed of Pf NF54 strain SPZ (15) was conducted in 80 adults (14), who received up to 6 doses of 1.35×10^5 PfSPZ Vaccine subcutaneously (SC) or intradermally (ID). The PfSPZ Vaccine was safe and well-tolerated, but elicited low-level immune responses and minimal protection. We hypothesized that the limited efficacy was due to the inefficiency of ID and SC administration for sufficient presentation of PfSPZ antigens at critical inductive sites required to achieve a protective immunological threshold (16). Therefore, we immunized non-human primates (NHPs) with the PfSPZ Vaccine and showed that intravenous (IV), but not SC administration of the PfSPZ Vaccine, elicited potent and durable PfSPZ-specific T-cell responses in peripheral blood and most notably in the liver (14), the likely site of immune protection (17). On the basis of these data, we

hypothesized that IV administration of the PfSPZ Vaccine would be protective in humans (14) and conducted a phase 1 clinical trial to determine safety, immunogenicity and protective efficacy against CHMI of IV immunization with the PfSPZ Vaccine (18).

Study Population

Fifty-seven subjects, composed of 40 vaccine recipients, 12 CHMI controls and 5 back-up controls, enrolled from October 2011 to October 2012 (table S1 shows baseline characteristics). 36/40 (90%) subjects completed all scheduled vaccinations (Fig. 1 and fig. S1). Of these 36 subjects, 2 were immunized with 2×10^3 PfSPZ Vaccine/dose to assess

safety only on schedules without CHMI, whereas at higher doses, CHMI was administered to 17 in July 2012 and to 15 in October 2012. Two subjects in the 1.35×10^5 PfSPZ Vaccine/dose group did not undergo CHMI due to an unrelated serious adverse event (SAE) and travel, respectively (Fig. 1).

Adverse Events

Vaccinations were well-tolerated and there were no breakthrough malaria infections prior to CHMI (Table 1). Thirty (75%) vaccine recipients had no local reactogenicity, 9 (22.5%) had mild pain/tenderness or bruising and 1 (2.5%) had moderate bruising at the injection site (Table 1 and table S2). Solicited systemic reactogenicity was none for 19 (47.5%), mild for 16 (40%), moderate for 4 (10%) and severe for 1 (2.5%) subject (Table 1 and table S3); the latter reported a headache after the third dose of 1.35×10^5 PfSPZ Vaccine.

Transient, asymptomatic increases in aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), assessed as possibly related to vaccination, were observed in 16 (40%) of vaccinees and were not dose-dependent (table S4 and figs. S2 and S3). Two SAEs occurred: one subject was diagnosed with colon cancer and did not participate in CHMI. After CHMI, one vaccine recipient developed anxiety and headache 14 days after completing chloroquine treatment, and was hospitalized overnight for diagnostic purposes. The symptoms, which were attributed to chloroquine, resolved within one month.

Vaccine Efficacy CHMI #1

In July 2012, subjects who received 7.5×10^3 and 3×10^4 PfSPZ Vaccine/dose underwent CHMI ~3 weeks after last immunization and 16 of 17 developed parasitemia (Fig. 2). Among the 9 subjects who had received 4 doses of 3×10^4 PfSPZ Vaccine, 1 did not develop parasitemia, whereas the other 8 had a 1.4 day prolongation of time to parasitemia by thick blood smear (pre-patent period) as compared to the 6 non-vaccinated controls ($P = 0.007$, Log-Rank) (Fig. 2 and table S5). This observation suggests a modest reduction in the numbers of liver stage parasites. The pre-patent periods in the 7.5×10^3 PfSPZ Vaccine/dose group were not significantly different than controls (Fig. 2 and table S5).

Vaccine Efficacy CHMI #2

In October 2012, subjects who received 1.35×10^5 PfSPZ Vaccine/dose underwent CHMI ~3 weeks after last immunization along with 6 new controls. Five of 6 controls developed parasitemia. Three of 9 subjects in the 4-dose group and none of 6 in the 5-dose group developed parasitemia ($P = 0.015$ for 5-dose group vs. controls, Fisher's exact test) (Fig. 2 and table S5). All subjects who did not develop parasitemia detected by thick blood smear were negative by quantitative polymerase chain reaction (qPCR) at 28 days after CHMI. In the three vaccinated subjects that became infected there was a modest delay in the time to positive PCR (table S5). Overall, 12 of 15 subjects immunized with 1.35×10^5 PfSPZ Vaccine/dose were protected ($P = 0.028$) (Fig. 2 and table S5).

Antibody Responses

Monoclonal antibodies against the major surface protein on SPZ, the circumsporozoite protein (CSP), prevent malaria by blocking SPZ invasion and development in hepatocytes (19). Furthermore, the RTS,S/AS01 malaria vaccine is thought to mediate protection primarily through induction of high levels of antibodies against PfCSP (20). Antibodies mediate some protection in rodents immunized with irradiated SPZ (21). In human subjects immunized via irradiated PfSPZ-infected mosquito bites, PfCSP or PfSPZ antibody titers have been detected by ELISA (enzyme-linked immunosorbent assay) or immunofluorescence assay (IFA), respectively (22). On the basis of these data, we assessed

antibodies against PfCSP and the entire PfSPZ (IFA), as well as performing a functional assay using subject serum to assess inhibition of sporozoite invasion (ISI) in a hepatocyte line in vitro.

Two weeks after the final vaccination there was a correlation between the total dosage of PfSPZ Vaccine administered and results of PfCSP ELISA, PfSPZ IFA, and PfSPZ ISI (Spearman $R = 0.77$, 0.83 and 0.84 respectively; $P < 0.001$) (Fig. 3, A to C). Among the 9 subjects who received 4 doses of 1.35×10^5 PfSPZ Vaccine, antibodies for all 3 assays were numerically higher in the 6 protected than the 3 non-protected subjects and significantly higher for the PfSPZ ISI ($P = 0.05$) (Fig. 3, A to C).

Antibodies to asexual or sexual erythrocytic stage parasites and antigens were undetectable, indicating that the irradiated PfSPZ were attenuated and did not develop beyond the early liver stage.

Cellular Immune Responses

Studies in mice and NHPs show that protective immunity induced by irradiated SPZ requires cellular immunity, and most pre-clinical data indicate that $CD8^+$ T-cells and interferon- γ (IFN- γ) are critical mediators of protection (21, 23–25). $CD4^+$ T-cells, $CD3^+ \gamma\delta$ T-cells, and natural killer (NK) cells can also play a role in protection (24, 26, 27). Therefore, we used multi-parameter flow cytometry to assess the frequency of PfSPZ-specific IFN- γ , interleukin (IL)-2, tumor necrosis factor (TNF) or perforin-producing $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+ \gamma\delta$ T-cells and NK cells from cryopreserved peripheral blood mononuclear cells (PBMCs) two weeks after the final immunization (fig. S4). The memory phenotype of PfSPZ-specific cells was assessed based on differential expression of the cell surface markers, CD45RA and CCR7 (fig. S4, C and G).

PfSPZ-specific, memory $CD3^+CD4^+$ T-cells from a representative protected subject produced IFN- γ , IL-2 and TNF, whereas PfSPZ-specific memory $CD3^+CD8^+$ T-cells produced IFN- γ , but little TNF and no IL-2 (Fig. 3D). There was a dose-dependent increase in frequency of PfSPZ-specific $CD3^+CD4^+$ T-cells ($P \leq 0.001$) and $CD3^+CD8^+$ T-cells producing any combination of IFN- γ , IL-2 or TNF ($P = 0.01$) (Fig. 3E).

We assessed PfSPZ-specific, IFN- γ -producing T-cells in subjects who received 1.35×10^5 PfSPZ Vaccine per injection, because of the critical role of IFN- γ in mediating attenuated SPZ-induced protective immunity. For PfSPZ-specific $CD3^+CD4^+$ IFN- γ producing T-cells, there was no significant difference between protected and unprotected subjects (Fig. 4A). For PfSPZ-specific $CD3^+CD8^+$ IFN- γ producing T-cells, protected subjects who received 4 doses, showed a trend toward higher and more consistent responses (Fig. 4A). Although all subjects who received 5 doses were protected, there was considerable variability in their responses. In terms of memory phenotype, PfSPZ-specific cytokine-producing $CD3^+CD4^+$ T-cells were detected in central (T_{CM}) and effector (T_{EM}) memory subsets (Fig. 4B and figs. S4G and S5). In contrast, PfSPZ-specific $CD3^+CD8^+$ T-cells were within effector (T_{EM}) and terminal effector (T_{TE}) memory subsets (Fig. 4B and fig. S4G).

We next characterized the quality of PfSPZ-specific T-cell cytokine responses. Amongst PfSPZ-specific $CD3^+CD4^+$ T-cells, there were three primary populations comprised of IFN- γ , IL-2 and TNF; IL-2 and TNF; or TNF (Fig. 4C) and no differences between protected and unprotected subjects. In contrast, among protected subjects, ~50–80% of the total PfSPZ-specific $CD3^+CD8^+$ T-cells produced IFN- γ only, as compared to <30% in unprotected subjects (Fig. 4D). The PfSPZ-specific IFN- γ producing $CD3^+CD8^+$ T-cells uniformly expressed perforin as did other effector lymphocyte populations except for $CD3^+CD4^+$ T-cells (fig. S4F).

$CD3^+ \gamma\delta$ T-cells from naïve humans respond to malaria antigens following in vitro stimulation (28). Accordingly, we also detected IFN- γ in $CD3^+ \gamma\delta$ T-cells from the majority of subjects prior to immunization in response to PfSPZ stimulation (fig. S4D). Although the proportion of $CD3^+ \gamma\delta$ T-cells producing IFN- γ in response to PfSPZ stimulation did

not change after vaccination (fig. S4E), the overall frequency of CD3⁺ γδ T-cells increased in subjects who received 1.35x10⁵ PfSPZ Vaccine/dose (Fig. 5).

Finally, to assess cellular immunity by a method commonly reported for other malaria vaccine trials, we used ELISpot assays to enumerate the frequency of IFN-γ responses to PfSPZ and six Pf proteins. PfSPZ-specific responses ranged from 100-300 SFC/10⁶ PBMCs and were highest in the 1.35 x 10⁵ PfSPZ Vaccine 5-dose group (fig. S6A). Responses to stimulation with peptide pools spanning five individual Pf pre-erythrocytic stage antigens were low to undetectable (mostly <100 SFC/10⁶ PBMCs) but generally higher than to one Pf blood-stage antigen included as a control (MSP1) (fig. S6B).

Discussion

The protection against infection we observed in all of the subjects who received the highest dosage of PfSPZ Vaccine has only been achieved previously by immunization with whole PfSPZ administered by mosquito bites (11, 29). The dose threshold at which we observed the highest rate of protection is consistent with prior studies in which >90% protection was associated with exposure to >1,000 irradiated PfSPZ-infected mosquitoes (11).

The 5-dose group in which all vaccinees were protected differed in two potentially important ways from other groups. These subjects received the highest total number of PfSPZ Vaccine (6.75 x 10⁵) and there was a 7-week interval between the fourth and fifth doses. We do not know if the protection in all subjects who received 5 doses compared to subjects who received 4 doses of 1.35 x 10⁵ PfSPZ Vaccine was due to the total dosage of PfSPZ, the numbers of doses or the increased interval between the 4th and 5th dose. Studies in NHPs show that extending the time between the 4th and 5th dose to ~8 weeks boosted the PfSPZ-specific CD8⁺ T-cell responses as compared to 4 weeks. Demonstrating that modifications in dose and schedule can achieve a protective immunological threshold in humans will guide future clinical trials designed to optimize the immunization regimen.

Two clinical trials of the PfSPZ Vaccine have established that IV administration of PfSPZ Vaccine induced superior immunogenicity and protective efficacy compared to SC and ID administration (14). The findings are consistent with prior studies in NHP showing that IV administration induced a higher frequency of liver resident CD8⁺ T-cells specific for PfSPZ and antibodies against PfSPZ (14). Although the IV route is routinely used for therapeutic interventions in humans, it has not previously been used for administration of preventive vaccines against infectious diseases. Accordingly, a series of clinical trials of the PfSPZ Vaccine administered by IV injection are now planned in Africa, Europe and the U.S. to expand critical data on the vaccine for clinical development as a method to prevent malaria in travelers, military, and other high-risk groups and for mass immunization of populations to eliminate Pf malaria from geographically defined areas. The findings of the current study suggest that the protective immune threshold could potentially be achieved in humans with higher doses administered by other routes, as has been done in mice (14). This could be facilitated by microneedle arrays or other novel devices.

The dose-dependent increase in the frequencies of PfSPZ-specific CD3+CD4+, CD3+CD8+ and CD3+γδ T-cells in the peripheral blood following immunization is consistent with studies of Pf infection and treatment (28). As CD3+γδ T-cells and other IFN-γ producing cells such as NK cells represent a higher proportion of lymphocytes in liver compared to blood in humans (30), they could contribute to protection.

Although we detected PfSPZ-specific CD3⁺CD8⁺ T-cells in 7 of 12 protected subjects at 1.35 x 10⁵ PfSPZ Vaccine/dose, 5 protected subjects had low to undetectable responses. This finding is consistent with data obtained from NHPs studies. We reported that some NHPs with a high frequency (~3%) of PfSPZ-specific CD3⁺CD8⁺ T-cells in the liver

had no detectable responses in blood following IV immunization with PfSPZ Vaccine (14). We speculate that as in mice (21, 23, 24) and NHPs (25), PfSPZ-specific CD3⁺CD8⁺ T-cells are required for protection in most individuals and are primarily confined to the liver due to persistence of parasite antigens following vaccination and/or retained as tissue-resident memory cells (31).

There was a dose-dependent increase in PfSPZ-specific antibodies using three assays. Although antibodies may have contributed to protection, such responses may best be used to predict vaccine take, and serve as a biomarker or non-mechanistic correlate of protection.

Vaccine efficacy against CHMI in this trial was considerably higher than that induced by subunit vaccines (6, 32) and consistent with data generated by immunization with mosquito bites (11). However, the antibody and cellular immune responses induced by the PfSPZ Vaccine against the specific malaria antigens tested were substantially lower than those induced by experimental Pf subunit vaccines (6, 32–35). It remains an open question whether protection results from the summation of multiple low level antigen-specific responses or from robust responses to a small number of as yet unidentified antigens from among the ~1,000 proteins expressed by sporozoites (36). We show that there are multiple populations of lymphocytes responding to PfSPZ providing a breadth of effector responses. Furthermore, we speculate that the PfSPZ Vaccine induces immunity to a broad spectrum of antigens among the ~1,000 expressed by attenuated PfSPZ (36–38). The capacity to induce a cascade of multiple adaptive and innate effector cells and the breadth of antigenic specificity may explain the advantage of whole PfSPZ vaccines compared to existing subunit vaccines.

Demonstration of high-level protection by IV administration is a critical first step in the development of the PfSPZ Vaccine. Future studies will determine the duration of protection and degree of protection against heterologous strains of Pf, establish immune correlates of protection, and optimize approaches to deliver IV vaccine administration to achieve the coverage in mass administration campaigns needed to eliminate Pf malaria from defined areas.

References and Notes

1. World Health Organization, World Malaria Report: 2012 (2012); www.who.int/malaria/publications/world_malaria_report_2012/repor t/en/index.html.
2. C. J. Murray, L. C. Rosenfeld, S. S. Lim, K. G. Andrews, K. J. Foreman, D. Haring, N. Fullman, M. Naghavi, R. Lozano, A. D. Lopez, Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* **379**, 413–431 (2012). [doi:10.1016/S0140-6736\(12\)60034-8](https://doi.org/10.1016/S0140-6736(12)60034-8) [Medline](#)
3. C. V. Plowe, P. Alonso, S. L. Hoffman, The potential role of vaccines in the elimination of falciparum malaria and the eventual eradication of malaria. *J. Infect. Dis.* **200**, 1646–1649 (2009). [doi:10.1086/646613](https://doi.org/10.1086/646613) [Medline](#)
4. malERA Consultative Group on Vaccines, A research agenda for malaria eradication: vaccines. *PLoS Med.* **8**, e1000398 (2011). [doi:10.1371/journal.pmed.1000398](https://doi.org/10.1371/journal.pmed.1000398) [Medline](#)
5. Malaria Vaccine Technology Roadmap, 2006; www.malaria vaccine.org/files/Malaria_Vaccine_TRM_Final.pdf
6. K. E. Kester, J. F. Cummings, O. Ofori-Anyinam, C. F. Ockenhouse, U. Krzych, P. Moris, R. Schwenk, R. A. Nielsen, Z. Debebe, E. Pinelis, L. Juompan, J. Williams, M. Dowler, V. A. Stewart, R. A. Wirtz, M. C. Dubois, M. Lievens, J. Cohen, W. R. Ballou, D. G. Heppner, Jr.; RTS,S Vaccine Evaluation Group, Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. *J. Infect. Dis.* **200**, 337–346 (2009). [doi:10.1086/600120](https://doi.org/10.1086/600120) [Medline](#)
7. S. T. Agnandji, B. Lell, J. F. Fernandes, B. P. Abooso, B. G. Methogo, A. L. Kabwende, A. A. Adegnik, B. Mordmüller, S. Issifou, P. G. Kremsner, J. Sacarlal, P. Aide, M. Lanasa, J. J. Aponte, S. Machevo, S. Acacio, H. Bulo, B. Sigauque, E. Macete, P.

- Alonso, S. Abdulla, N. Salim, R. Minja, M. Mpina, S. Ahmed, A. M. Ali, A. T. Mtoro, A. S. Hamad, P. Mutani, M. Tanner, H. Tinto, U. D'Alessandro, H. Sorgho, I. Valea, B. Bihoun, I. Guiraud, B. Kaboré, O. Sombié, R. T. Guiguemdé, J. B. Ouédraogo, M. J. Hamel, S. Kariuki, M. Oneko, C. Odero, K. Otieno, N. Awino, M. McMorrow, V. Muturi-Kioi, K. F. Laserson, L. Slutsker, W. Otieno, L. Otieno, N. Otsyula, S. Gondi, A. Otieno, V. Owira, E. Oguk, G. Odongo, J. B. Woods, B. Ogutu, P. Njuguna, R. Chilengi, P. Akoo, C. Kerubo, C. Maingi, T. Lang, A. Olotu, P. Bejon, K. Marsh, G. Mwambingu, S. Owusu-Agyei, K. P. Asante, K. Osei-Kwakye, O. Boahen, D. Dosoo, I. Asante, G. Adjei, E. Kwara, D. Chandramohan, B. Greenwood, J. Lusingu, S. Gesase, A. Malabeja, O. Abdul, C. Mahende, E. Liheluka, L. Malle, M. Lemnge, T. G. Theander, C. Drakeley, D. Ansong, T. Agbenyega, S. Adjei, H. O. Boateng, T. Rettig, J. Bawa, J. Sylverken, D. Sambian, A. Sarfo, A. Agyekum, F. Martinson, I. Hoffman, T. Mvalo, P. Kamthunzi, R. Nkomo, T. Tembo, G. Tegha, M. Tsidy, J. Kilembe, C. Chawinga, W. R. Ballou, J. Cohen, Y. Guerra, E. Jongert, D. Lapiere, A. Leach, M. Lievens, O. Ofori-Anyinam, A. Olivier, J. Vekemans, T. Carter, D. Kaslow, D. Lebouilleux, C. Loucq, A. Radford, B. Savarese, D. Schellenberg, M. Sillman, P. Vansadia; RTS,S Clinical Trials Partnership, A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N. Engl. J. Med.* **367**, 2284–2295 (2012). [doi:10.1056/NEJMoa1208394](https://doi.org/10.1056/NEJMoa1208394) [Medline](#)
8. R. S. Nussenzweig, J. P. Vanderberg, H. Most, C. Orton, Specificity of protective immunity produced by x-irradiated *Plasmodium berghei* sporozoites. *Nature* **222**, 488–489 (1969). [doi:10.1038/222488a0](https://doi.org/10.1038/222488a0) [Medline](#)
9. D. F. Clyde, H. Most, V. C. McCarthy, J. P. Vanderberg, Immunization of man against sporozite-induced falciparum malaria. *Am. J. Med. Sci.* **266**, 169–177 (1973). [doi:10.1097/00000441-197309000-00002](https://doi.org/10.1097/00000441-197309000-00002) [Medline](#)
10. K. H. Rieckmann, P. E. Carson, R. L. Beaudoin, J. S. Cassells, K. W. Sell, Letter: Sporozoite induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* **68**, 258–259 (1974). [doi:10.1016/0035-9203\(74\)90129-1](https://doi.org/10.1016/0035-9203(74)90129-1) [Medline](#)
11. S. L. Hoffman, L. M. Goh, T. C. Luke, I. Schneider, T. P. Le, D. L. Doolan, J. Sacci, P. de la Vega, M. Dowler, C. Paul, D. M. Gordon, J. A. Stoute, L. W. Church, M. Sedegah, D. G. Heppner, W. R. Ballou, T. L. Richie, Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. *J. Infect. Dis.* **185**, 1155–1164 (2002). [doi:10.1086/339409](https://doi.org/10.1086/339409) [Medline](#)
12. T. C. Luke, S. L. Hoffman, Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. *J. Exp. Biol.* **206**, 3803–3808 (2003). [doi:10.1242/jeb.00644](https://doi.org/10.1242/jeb.00644) [Medline](#)
13. S. L. Hoffman, P. F. Billingsley, E. James, A. Richman, M. Loyevsky, T. Li, S. Chakravarty, A. Gunasekera, R. Chattopadhyay, M. Li, R. Stafford, A. Ahumada, J. E. Epstein, M. Sedegah, S. Reyes, T. L. Richie, K. E. Lyke, R. Edelman, M. B. Laurens, C. V. Plowe, B. K. Sim, Development of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria. *Hum. Vaccin.* **6**, 97–106 (2010). [doi:10.4161/hv.6.1.10396](https://doi.org/10.4161/hv.6.1.10396) [Medline](#)
14. J. E. Epstein, K. Tewari, K. E. Lyke, B. K. Sim, P. F. Billingsley, M. B. Laurens, A. Gunasekera, S. Chakravarty, E. R. James, M. Sedegah, A. Richman, S. Velmurugan, S. Reyes, M. Li, K. Tucker, A. Ahumada, A. J. Ruben, T. Li, R. Stafford, A. G. Eappen, C. Tamminga, J. W. Bennett, C. F. Ockenhouse, J. R. Murphy, J. Komisar, N. Thomas, M. Loyevsky, A. Birkett, C. V. Plowe, C. Loucq, R. Edelman, T. L. Richie, R. A. Seder, S. L. Hoffman, Live attenuated malaria vaccine designed to protect through hepatic CD8⁺ T cell immunity. *Science* **334**, 475–480 (2011). [doi:10.1126/science.1211548](https://doi.org/10.1126/science.1211548) [Medline](#)
15. T. Ponnudurai, J. H. Meuwissen, A. D. Leeuwenberg, J. P. Verhave, A. H. Lensen, The production of mature gametocytes of *Plasmodium falciparum* in continuous cultures of different isolates infective to mosquitoes. *Trans. R. Soc. Trop. Med. Hyg.* **76**, 242–250 (1982). [doi:10.1016/0035-9203\(82\)90289-9](https://doi.org/10.1016/0035-9203(82)90289-9) [Medline](#)
16. S. Chakravarty, I. A. Cockburn, S. Kuk, M. G. Overstreet, J. B. Sacci, F. Zavala, CD8⁺ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. *Nat. Med.* **13**, 1035–1041 (2007). [doi:10.1038/nm1628](https://doi.org/10.1038/nm1628) [Medline](#)
17. S. L. Hoffman, D. L. Doolan, Malaria vaccines-targeting infected hepatocytes. *Nat. Med.* **6**, 1218–1219 (2000). [doi:10.1038/81315](https://doi.org/10.1038/81315) [Medline](#)
18. Materials and methods are available as supplementary material on Science Online.
19. P. Potocnjak, N. Yoshida, R. S. Nussenzweig, V. Nussenzweig, Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *J. Exp. Med.* **151**, 1504–1513 (1980). [doi:10.1084/jem.151.6.1504](https://doi.org/10.1084/jem.151.6.1504) [Medline](#)
20. V. S. Moorthy, W. R. Ballou, Immunological mechanisms underlying protection mediated by RTS,S: a review of the available data. *Malar. J.* **8**, 312 (2009). [doi:10.1186/1475-2875-8-312](https://doi.org/10.1186/1475-2875-8-312) [Medline](#)
21. L. Schofield, J. Villaquiran, A. Ferreira, H. Schellekens, R. Nussenzweig, V. Nussenzweig, Gamma interferon, CD8⁺ T cells and antibodies required for immunity to malaria sporozoites. *Nature* **330**, 664–666 (1987). [doi:10.1038/330664a0](https://doi.org/10.1038/330664a0) [Medline](#)
22. J. E. Egan, S. L. Hoffman, J. D. Haynes, J. C. Sadoff, I. Schneider, G. E. Grau, M. R. Hollingdale, W. R. Ballou, D. M. Gordon, Humoral immune responses in volunteers immunized with irradiated *Plasmodium falciparum* sporozoites. *Am. J. Trop. Med. Hyg.* **49**, 166–173 (1993). [Medline](#)
23. W. R. Weiss, M. Sedegah, R. L. Beaudoin, L. H. Miller, M. F. Good, CD8⁺ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 573–576 (1988). [doi:10.1073/pnas.85.2.573](https://doi.org/10.1073/pnas.85.2.573) [Medline](#)
24. D. L. Doolan, S. L. Hoffman, The complexity of protective immunity against liver-stage malaria. *J. Immunol.* **165**, 1453–1462 (2000). [Medline](#)
25. W. R. Weiss, C. G. Jiang, Protective CD8⁺ T lymphocytes in primates immunized with malaria sporozoites. *PLoS ONE* **7**, e31247 (2012). [doi:10.1371/journal.pone.0031247](https://doi.org/10.1371/journal.pone.0031247) [Medline](#)
26. W. R. Weiss, M. Sedegah, J. A. Berzofsky, S. L. Hoffman, The role of CD4⁺ T cells in immunity to malaria sporozoites. *J. Immunol.* **151**, 2690–2698 (1993). [Medline](#)
27. M. Tsuji, P. Mombaerts, L. Lefrancois, R. S. Nussenzweig, F. Zavala, S. Tonegawa, Gamma delta T cells contribute to immunity against the liver stages of malaria in alpha beta T-cell-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 345–349 (1994). [doi:10.1073/pnas.91.1.345](https://doi.org/10.1073/pnas.91.1.345) [Medline](#)
28. A. C. Teirlinck, M. B. McCall, M. Roestenberg, A. Scholzen, R. Woestenenq, Q. de Mast, A. J. van der Ven, C. C. Hermsen, A. J. Luty, R. W. Sauerwein, Longevity and composition of cellular immune responses following experimental *Plasmodium falciparum* malaria infection in humans. *PLoS Pathog.* **7**, e1002389 (2011). [doi:10.1371/journal.ppat.1002389](https://doi.org/10.1371/journal.ppat.1002389) [Medline](#)
29. M. Roestenberg, M. McCall, J. Hopman, J. Wiersma, A. J. Luty, G. J. van Gemert, M. van de Vegte-Bolmer, B. van Schaijk, K. Teelen, T. Arens, L. Spaarman, Q. de Mast, W. Roeffen, G. Snounou, L. Rénia, A. van der Ven, C. C. Hermsen, R. Sauerwein, Protection against a malaria challenge by sporozoite inoculation. *N. Engl. J. Med.* **361**, 468–477 (2009). [doi:10.1056/NEJMoa0805832](https://doi.org/10.1056/NEJMoa0805832) [Medline](#)
30. D. G. Doherty, C. O'Farrelly, Innate and adaptive lymphoid cells in the human liver. *Immunol. Rev.* **174**, 5–20 (2000). [doi:10.1034/j.1600-0528.2002.017416.x](https://doi.org/10.1034/j.1600-0528.2002.017416.x) [Medline](#)
31. I. A. Cockburn, Y. C. Chen, M. G. Overstreet, J. R. Lees, N. van Rooijen, D. L. Farber, F. Zavala, Prolonged antigen presentation is required for optimal CD8⁺ T cell responses against malaria liver stage parasites. *PLoS Pathog.* **6**, e1000877 (2010). [doi:10.1371/journal.ppat.1000877](https://doi.org/10.1371/journal.ppat.1000877) [Medline](#)
32. I. Chuang, M. Sedegah, S. Cicatelli, M. Spring, M. Polhemus, C. Tamminga, N. Patterson, M. Guerrero, J. W. Bennett, S. McGrath, H. Ganeshan, M. Belmonte, F. Farooq, E. Abot, J. G. Banania, J. Huang, R. Newcomer, L. Rein, D. Lilit, N. O. Richie, C. Wood, J. Murphy, R. Sauerwein, C. C. Hermsen, A. J. McCoy, E. Kamau, J.

- Cummings, J. Komisar, A. Sutamihardja, M. Shi, J. E. Epstein, S. Maiolatesi, D. Tosh, K. Limbach, E. Angov, E. Bergmann-Leitner, J. T. Bruder, D. L. Doolan, C. R. King, D. Carucci, S. Dutta, L. Soisson, C. Diggs, M. R. Hollingdale, C. F. Ockenhouse, T. L. Richie, DNA prime/Adenovirus boost malaria vaccine encoding *P. falciparum* CSP and AMA1 induces sterile protection associated with cell-mediated immunity. *PLoS ONE* **8**, e55571 (2013). [doi:10.1371/journal.pone.0055571](https://doi.org/10.1371/journal.pone.0055571) [Medline](#)
33. J. A. Stoute, M. Slaoui, D. G. Heppner, P. Momin, K. E. Kester, P. Desmons, B. T. Wellde, N. Garçon, U. Krzych, M. Marchand, W. R. Ballou, J. D. Cohen, A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. RTS,S Malaria Vaccine Evaluation Group. *N. Engl. J. Med.* **336**, 86–91 (1997). [doi:10.1056/NEJM199701093360202](https://doi.org/10.1056/NEJM199701093360202) [Medline](#)
34. K. E. Kester, D. A. McKinney, N. Tornieporth, C. F. Ockenhouse, D. G. Heppner, T. Hall, U. Krzych, M. Delchambre, G. Voss, M. G. Dowler, J. Palensky, J. Wittes, J. Cohen, W. R. Ballou, RTS,S Malaria Vaccine Evaluation Group, Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. *J. Infect. Dis.* **183**, 640–647 (2001). [doi:10.1086/318534](https://doi.org/10.1086/318534) [Medline](#)
35. G. A. O'Hara, C. J. Duncan, K. J. Ewer, K. A. Collins, S. C. Elias, F. D. Halstead, A. L. Goodman, N. J. Edwards, A. Reyes-Sandoval, P. Bird, R. Rowland, S. H. Sheehy, I. D. Poulton, C. Hutchings, S. Todryk, L. Andrews, A. Folgori, E. Berrie, S. Moyle, A. Nicosia, S. Colloca, R. Cortese, L. Siani, A. M. Lawrie, S. C. Gilbert, A. V. Hill, Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. *J. Infect. Dis.* **205**, 772–781 (2012). [doi:10.1093/infdis/jir850](https://doi.org/10.1093/infdis/jir850) [Medline](#)
36. L. Florens, M. P. Washburn, J. D. Raine, R. M. Anthony, M. Grainger, J. D. Haynes, J. K. Moch, N. Muster, J. B. Sacci, D. L. Tabb, A. A. Witney, D. Wolters, Y. Wu, M. J. Gardner, A. A. Holder, R. E. Sinden, J. R. Yates, D. J. Carucci, A proteomic view of the *Plasmodium falciparum* life cycle. *Nature* **419**, 520–526 (2002). [doi:10.1038/nature01107](https://doi.org/10.1038/nature01107) [Medline](#)
37. A. Trieu, M. A. Kayala, C. Burk, D. M. Molina, D. A. Freilich, T. L. Richie, P. Baldi, P. L. Felgner, D. L. Doolan, Sterile protective immunity to malaria is associated with a panel of novel *P. falciparum* antigens. *Mol. Cell. Proteomics* **10**, M11.007948 (2011). [doi:10.1074/mcp.M111.007948](https://doi.org/10.1074/mcp.M111.007948) [Medline](#)
38. D. L. Doolan, S. Southwood, D. A. Freilich, J. Sidney, N. L. Graber, L. Shatney, L. Bebris, L. Florens, C. Dobano, A. A. Witney, E. Appella, S. L. Hoffman, J. R. Yates, 3rd, D. J. Carucci, A. Sette, Identification of *Plasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9952–9957 (2003). [doi:10.1073/pnas.1633254100](https://doi.org/10.1073/pnas.1633254100) [Medline](#)
39. U. S. Food and Drug Administration, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. (2007; www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074775.htm).
40. J. D. Chulay, I. Schneider, T. M. Cosgriff, S. L. Hoffman, W. R. Ballou, I. A. Quakyi, R. Carter, J. H. Trosper, W. T. Hockmeyer, Malaria transmitted to humans by mosquitoes infected from cultured *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **35**, 66–68 (1986). [Medline](#)
41. L. S. Rickman, T. R. Jones, G. W. Long, S. Paparello, I. Schneider, C. F. Paul, R. L. Beaudoin, S. L. Hoffman, *Plasmodium falciparum*-infected *Anopheles stephensi* inconsistently transmit malaria to humans. *Am. J. Trop. Med. Hyg.* **43**, 441–445 (1990). [Medline](#)
42. K. E. Lyke, M. Laurens, M. Adams, P. F. Billingsley, A. Richman, M. Loyevsky, S. Chakravarty, C. V. Plowe, B. K. Sim, R. Edelman, S. L. Hoffman, *Plasmodium falciparum* malaria challenge by the bite of aseptic *Anopheles stephensi* mosquitoes: results of a randomized infectivity trial. *PLoS ONE* **5**, e13490 (2010). [doi:10.1371/journal.pone.0013490](https://doi.org/10.1371/journal.pone.0013490) [Medline](#)
43. D. Walliker, I. A. Quakyi, T. E. Welles, T. F. McCutchan, A. Szarfman, W. T. London, L. M. Corcoran, T. R. Burkot, R. Carter, Genetic analysis of the human malaria parasite *Plasmodium falciparum*. *Science* **236**, 1661–1666 (1987). [doi:10.1126/science.3299700](https://doi.org/10.1126/science.3299700) [Medline](#)
44. S. H. Sheehy, C. J. Duncan, S. C. Elias, P. Choudhary, S. Biswas, F. D. Halstead, K. A. Collins, N. J. Edwards, A. D. Douglas, N. A. Anagnostou, K. J. Ewer, T. Havelock, T. Mahungu, C. M. Bliss, K. Miura, I. D. Poulton, P. J. Lillie, R. D. Antrobus, E. Berrie, S. Moyle, K. Gantlett, S. Colloca, R. Cortese, C. A. Long, R. E. Sinden, S. C. Gilbert, A. M. Lawrie, T. Doherty, S. N. Faust, A. Nicosia, A. V. Hill, S. J. Draper, ChAd63-MVA-vectored blood-stage malaria vaccines targeting MSP1 and AMA1: assessment of efficacy against mosquito bite challenge in humans. *Mol. Ther.* **20**, 2355–2368 (2012). [doi:10.1038/mt.2012.223](https://doi.org/10.1038/mt.2012.223) [Medline](#)
45. Plasmodium 100 Genomes initiative, Broad Institute (https://olive.broadinstitute.org/genomes/plas_falc_nf4_v1.1).
46. M. J. Gardner, N. Hall, E. Fung, O. White, M. Berriman, R. W. Hyman, J. M. Carlton, A. Pain, K. E. Nelson, S. Bowman, I. T. Paulsen, K. James, J. A. Eisen, K. Rutherford, S. L. Salzberg, A. Craig, S. Kyes, M. S. Chan, V. Nene, S. J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M. W. Mather, A. B. Vaidya, D. M. Martin, A. H. Fairlamb, M. J. Fraunholz, D. S. Roos, S. A. Ralph, G. I. McFadden, L. M. Cummings, G. M. Subramanian, C. Mungall, J. C. Venter, D. J. Carucci, S. L. Hoffman, C. Newbold, R. W. Davis, C. M. Fraser, B. Barrell, Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511 (2002). [doi:10.1038/nature01097](https://doi.org/10.1038/nature01097) [Medline](#)
47. J. I. Cohen, G. Fahle, M. A. Kemp, K. Apakupakul, T. P. Margolis, Human herpesvirus 6-A, 6-B, and 7 in vitreous fluid samples. *J. Med. Virol.* **82**, 996–999 (2010). [doi:10.1002/jmv.21751](https://doi.org/10.1002/jmv.21751) [Medline](#)
48. S. E. Shokoples, M. Ndao, K. Kowalewska-Grochowska, S. K. Yanow, Multiplexed real-time PCR assay for discrimination of *Plasmodium* species with improved sensitivity for mixed infections. *J. Clin. Microbiol.* **47**, 975–980 (2009). [doi:10.1128/JCM.01858-08](https://doi.org/10.1128/JCM.01858-08) [Medline](#)
49. J. Sattabongkot, N. Yimamnuaychoke, S. Leelaudomlipi, M. Rasameesoraj, R. Jenwithisuk, R. E. Coleman, R. Udomsangpetch, L. Cui, T. G. Brewer, Establishment of a human hepatocyte line that supports in vitro development of the exo-erythrocytic stages of the malaria parasites *Plasmodium falciparum* and *P. vivax*. *Am. J. Trop. Med. Hyg.* **74**, 708–715 (2006). [Medline](#)
50. L. Rénia, F. Miltgen, Y. Charoenvit, T. Ponnudurai, J. P. Verhave, W. E. Collins, D. Mazier, Malaria sporozoite penetration. A new approach by double staining. *J. Immunol. Methods* **112**, 201–205 (1988). [doi:10.1016/0022-1759\(88\)90358-4](https://doi.org/10.1016/0022-1759(88)90358-4) [Medline](#)
51. M. Roederer, J. L. Nozzi, M. C. Nason, SPICE: exploration and analysis of post-cytometric complex multivariate datasets. *Cytometry A* **79A**, 167–174 (2011). [doi:10.1002/cyto.a.21015](https://doi.org/10.1002/cyto.a.21015) [Medline](#)
- Acknowledgments:** The data presented in this manuscript are tabulated in the main figures and the supplementary materials. The Clinical trial was funded and supported by the NIAID Intramural Research Program. Production and characterization of the vaccine were supported in part by NIAID SBIR grants, 4R44AI055229-08, 3R44AI055229-06S1, and 5R44AI058499-05. Pre-clinical toxicology and bio-distribution studies were supported in part by NIAID preclinical service contract N01-AI-40096. Molecular diagnosis was supported by the Howard Hughes Medical Institute. A materials transfer agreement will be required for the use of recombinant PfMSP-1 and PfEBA-175, HC-04 cells, and 2A10 monoclonal antibody. A number of patents on PfSPZ have been issued, allowed, or filed in the United States and internationally. The U.S. patents include S. L. Hoffman *et al.*, U.S. Patent 7,229,627 (2007) (there is a divisional of this patent with claims directed to aseptic adult *Anopheles*-species mosquitoes and aseptic *Plasmodium*-species sporozoites, USSN 11/726,622); S. L. Hoffman *et al.*, U.S. Patent Pub. US2005/0208078 (2005); and B. K. L. Sim, S. L. Hoffman, M. Li, R. E. Stafford, U.S. Patent Pub U.S. 2010/0183680 (2010). There is also a patent on HC-04 cells [J. Prachumsri *et al.*, U.S. Patent 7015036 (2006)]. The findings and conclusions in this report are those of the authors and do not necessarily reflect the

views of the funding agency or collaborators. The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, the Department of the Army, Department of Defense, nor the U.S. Government. This work was supported by work unit number 6000.RADI.F.A0309. The study protocol was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. Judith E. Epstein, Thomas L. Richie, Jason H. Richardson, Jittawadee Murphy, and Silas A. Davidson are military service members. This work was prepared as part of their official duties. Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Departments of the Army, Navy or Defense. The authors thank the vaccine trials participants for their contribution and commitment to vaccine research. We acknowledge the contributions of our NIH Clinical Center and NIAID colleagues, especially: Dr. A.S. Fauci for thoughtful advice and review of the manuscript, J. Stein, J. Pierson, R. Eckes, P. Driscoll, L. Ediger, and the nursing staff of the 5NW, SCSU, 5SE-S, and 5SE-N units; our VRC colleagues, especially: B. Flynn, A. Mittelman, M. Young, C. Artis, R. Hicks and T. Abram; the EMMES Corporation; R. Thompson, F. Beams, M. Garley, A. Hoffman and D. Dolberg of Sanaria Inc. for administrative, operations and legal support and T. Luke for insight and inspiration; the NIAID Institutional Review Board; the NIAID Office of Communications and Government Relations; the NIH Clinical Center IND Pharmacy and the NIH Clinical Center Patient Recruitment and Public Liaison Office. We appreciate the expert reviews of the Safety Monitoring Committee (A. Durbin, K. Kester and A. Cross) and the assistance from the U.S. Military Malaria Vaccine Program, the Walter Reed Army Institute of Research Entomology Branch and the Naval Medical Research Center, especially A. Reyes, Y. Alcorta, G. Banania, C. Fedders, M. Dowler, T. Savransky, D. Patel, C. Brando and K. Kobylnski.

Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1241800/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S6

Tables S1 to S6

References (39–51)

11 June 2013; accepted 25 July 2013

Published online 8 August 2013

10.1126/science.1241800

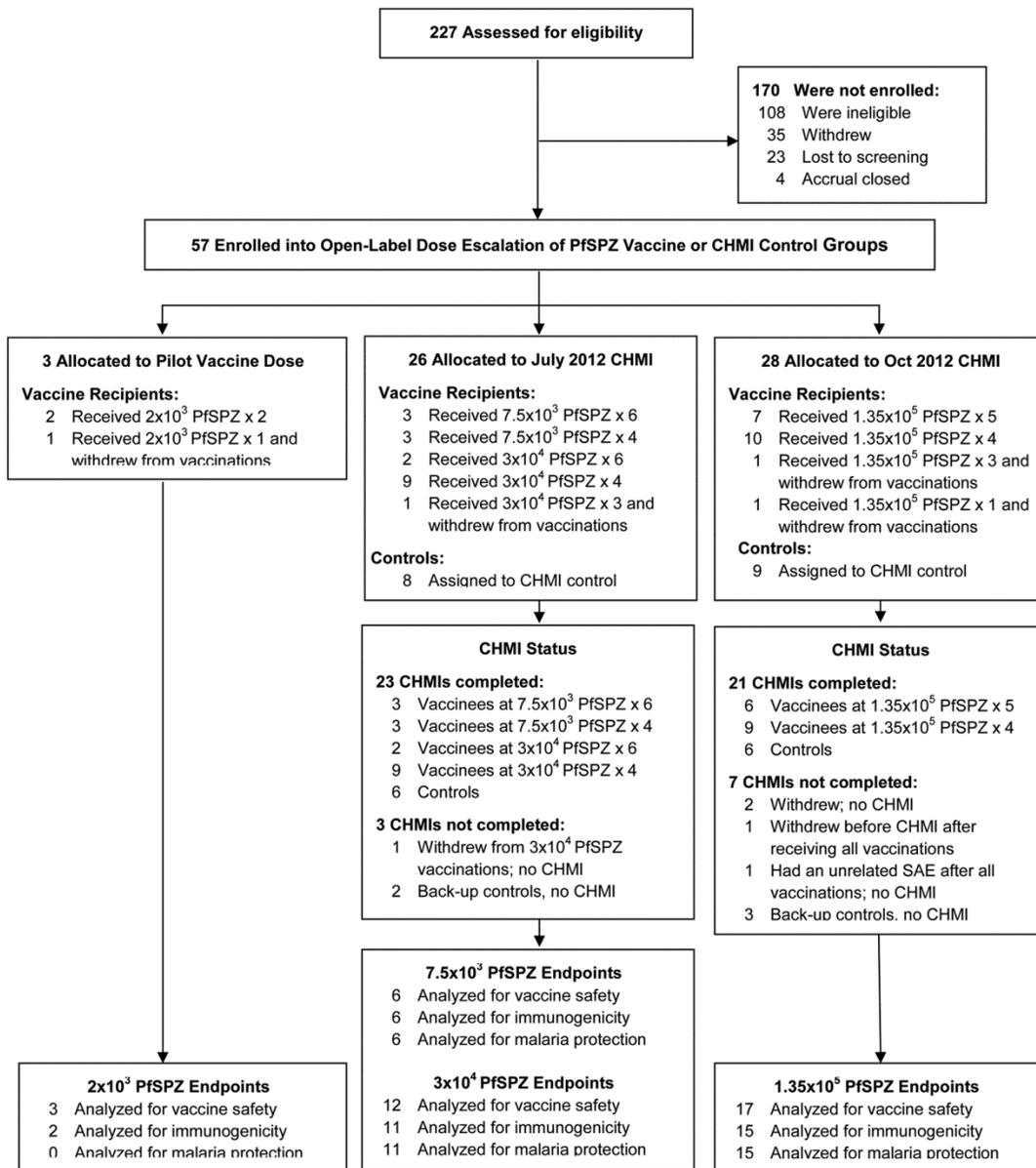
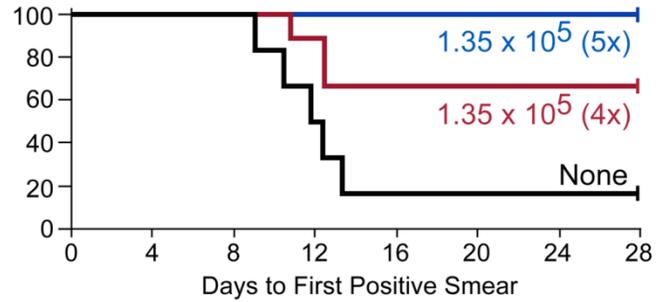
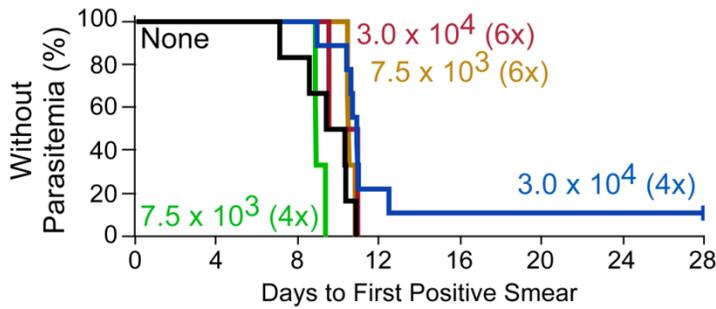


Fig. 1. Screening, enrollment, vaccinations, controlled human malaria infection (CHMI) and study endpoints analyses. SAE denotes serious adverse event.



Vaccination Dose	# Inj.	CHMI Parasite*	# of Subjects	Parasite Free	Vaccine Efficacy
None		NF54	6	0	
7.5×10^3	4	NF54	3	0	0%
7.5×10^3	6	NF54	3	0	0%
3.0×10^4	4	NF54	9	1	11%
3.0×10^4	6	NF54	2	0	0%

Vaccination Dose	# Inj.	CHMI Parasite*	# of Subjects	Parasite Free	Vaccine Efficacy
None		3D7	6	1	
1.35×10^5	4	3D7	9	6	60%
1.35×10^5	5	3D7	6	6	100%

Fig. 2. Kaplan-Meier curves and protection table results. Kaplan-Meier curves are shown for the July and October Controlled Human Malaria Infections (CHMIs) indicating the frequency of subjects who remained without *Plasmodium falciparum* parasitemia after CHMI. *CHMI Parasite refers to the Pf parasite used to produce infected mosquitoes for CHMI (see Supplementary Materials). Each dose group and regimen are labeled and listed in the tables. Vaccine efficacy (VE) against acquiring *P. falciparum* malaria is shown in the last column. For the groups receiving the 1.35×10^5 dose in the October CHMI, estimates of VE (defined as $1 - \text{Relative Risk}$), and (exact, unconditional) 95% Confidence Intervals (CIs) are as follows: 5 doses: 0/6 vs. 5/6 estimated RR = 0; estimated VE =100% (95% CI for VE [0.464, 1]); 4 doses: 3/9 vs. 5/6 estimated RR = 0.4; estimated VE=60% (95% CI for VE [-0.127, 0.92]); Combined: 3/15 vs. 5/6 estimated RR = 0.24; estimated VE=76% (95% CI for VE [0.272, 0.955]).

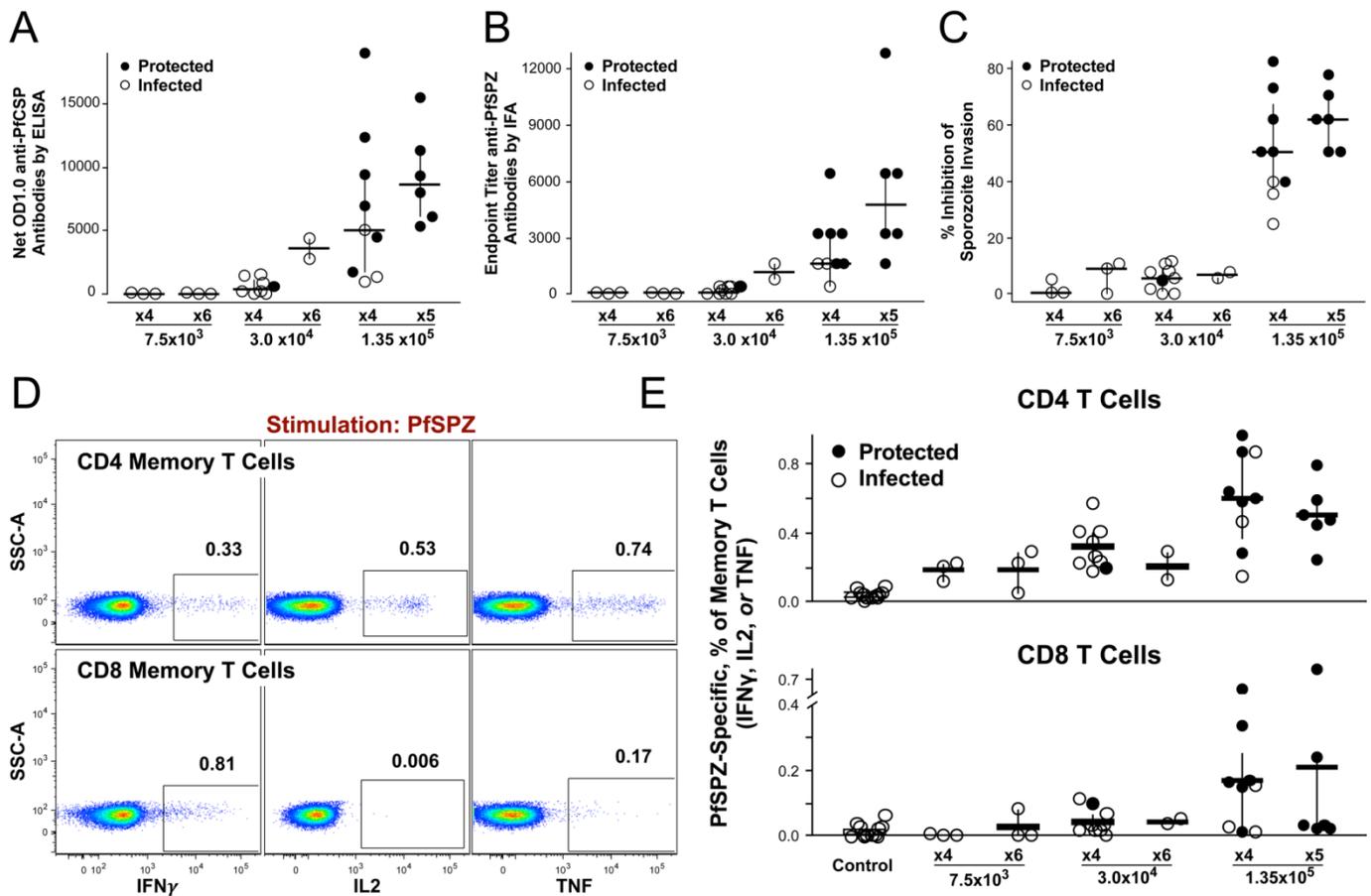


Fig. 3. Antibody, CD3+CD4+, and CD3+CD8+ T-cell cytokine responses in all immunized participants. (A to C) Antibodies. Assessment of antibodies was performed from serum of all subjects prior to immunization and 2 weeks after the last dose of the PfSPZ Vaccine, which was ~1 week prior to CHMI. (A) Net OD 1.0 anti-PfCSP antibodies by ELISA was the serum dilution for each volunteer at which the optical density was 1.0 based on the difference between values in post- and pre-immunization serum. (B) Endpoint titer antibodies to PfSPZ by IFA for each volunteer was the last serum dilution at which the IFA was positive. (C) Percent inhibition of sporozoite invasion was the percent reduction of the numbers of PfSPZ that invaded a human hepatocyte line in the presence of immune serum as compared to in the presence of pre-immunization serum from the same volunteer, both at a dilution of 1:5. (D and E) T-cells. PBMCs were analyzed by multi-parameter flow cytometry for PfSPZ-specific cytokine producing (IFN- γ , IL-2 or TNF) memory CD3+CD4+ and CD3+CD8+ T-cells from all subjects at week 2 after the final immunization with PfSPZ Vaccine following in vitro re-stimulation (fig. S4). The frequency of PfSPZ-specific memory CD3+CD4+ and CD3+CD8+ T-cells was calculated as the fraction of cells making any cytokine in response to PfSPZ minus the response to 1% HSA (diluent). (D) A representative dot plot showing PfSPZ-specific IFN- γ , IL-2 or TNF producing memory CD3+CD4+ (upper panel) and CD3+CD8+ (lower panel) T-cells from a volunteer who received 5 doses of 1.35x10⁵ PfSPZ Vaccine and was protected. (E) The frequency of total PfSPZ-specific cytokine producing (IFN- γ , IL-2 or TNF) memory CD3+CD4+ (upper panel) and CD3+CD8+ (lower panel) T-cells from all subjects within each group. Data in all four graphs show a point for each subject. Horizontal bars denote the medians and whiskers denote the inter-quartile ranges.

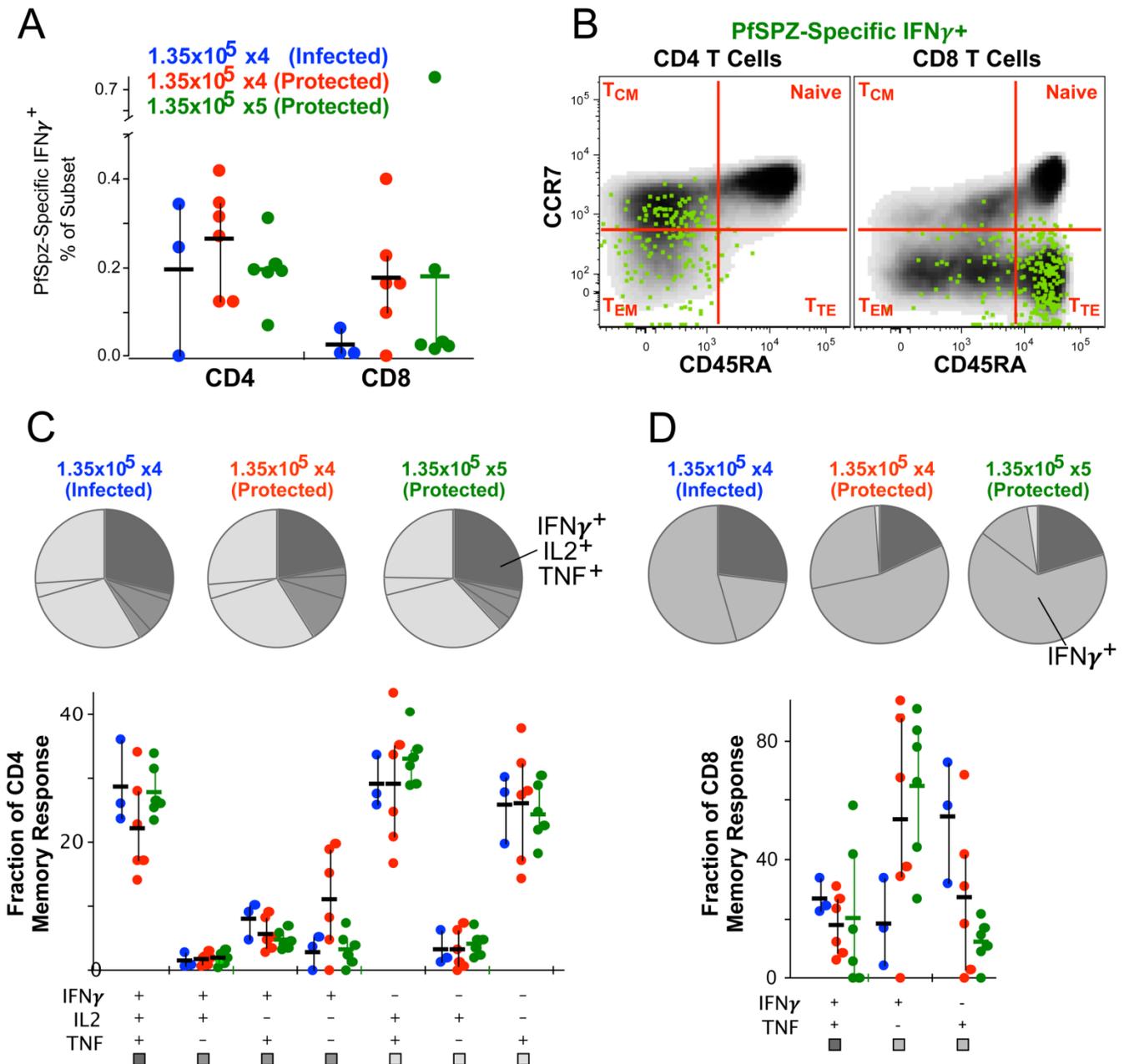


Fig. 4. Magnitude and quality of PfSPZ-specific memory T-cell cytokine responses in participants immunized with 1.35×10^5 PfSPZ vaccine/dose. PBMCs were analyzed by multi-parameter flow cytometry after *in vitro* stimulation with PfSPZ in all volunteers at week-2 post administration of final dose of 1.35×10^5 PfSPZ Vaccine (fig. S4). **(A)** The frequency of IFN- γ producing memory CD3+CD4+ or CD3+CD8+ T-cells in all protected and unprotected subjects who received 1.35×10^5 PfSPZ Vaccine either 4 or 5 times. **(B)** A representative dot plot showing the distribution of PfSPZ-specific IFN- γ producing CD3+CD4 and CD3+CD8+ T-cells within T_{CM}, T_{EM} and T_{TE} phenotype T-cells as defined by expression of CCR7 and CD45RA from a volunteer who received 5 doses of 1.35×10^5 PfSPZ Vaccine. The quality of PfSPZ-specific memory CD3+CD4+ **(C)** and CD3+CD8+ **(D)** T-cell response was determined using SPICE analysis, defining distinct populations producing any combination of IFN- γ , IL-2 or TNF (CD3+CD4+) or any combination of IFN- γ or TNF (CD3+CD8+) at the single cell level. The relative proportions of each of these populations from the three unprotected and 6 protected subjects who received 4 or 5 doses of 1.35×10^5 PfSPZ Vaccine is shown by pie charts or plotted as a percentage of the total cytokine response. In **(A)**, **(C)**, and **(D)**, a point is shown for each subject. Horizontal bars denote the medians and whiskers denote the inter-quartile ranges.

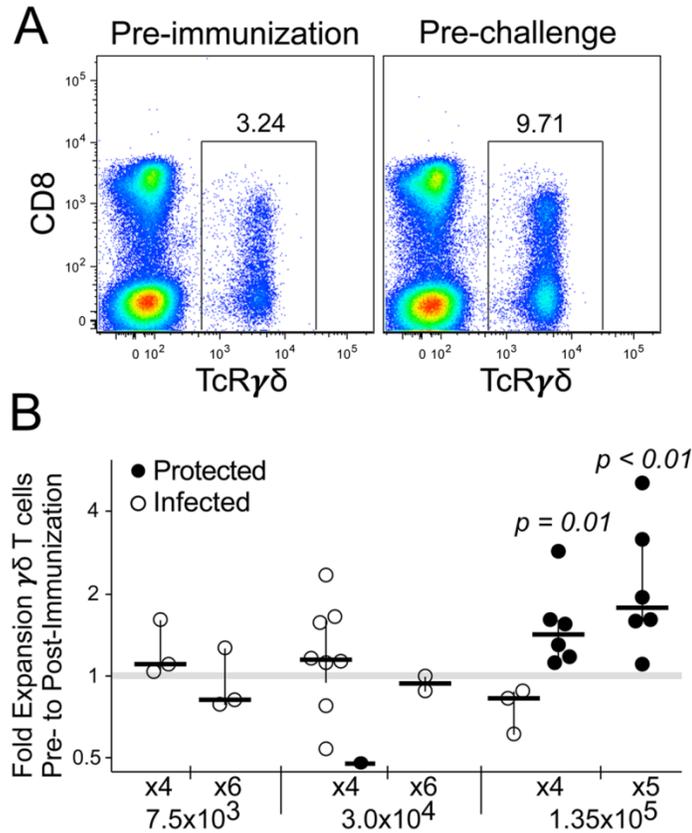


Fig. 5. Expansion of TCR $\gamma\delta$ T-cells following immunization with PfSPZ vaccine. PBMCs were analyzed for frequency of TCR $\gamma\delta$ T-cells in all subjects immunized with PfSPZ Vaccine pre-immunization and pre-CHMI as shown in fig. S4. **(A)** A representative dot plot showing the frequency of TCR $\gamma\delta$ T-cells pre-immunization and pre-CHMI from a volunteer immunized with 5 doses of 1.35×10^5 PfSPZ Vaccine. **(B)** The fold expansion in the total frequency of TCR $\gamma\delta$ T-cells following immunization with PfSPZ Vaccine is shown for subjects from all groups and distinguishes protected from non-protected volunteers. In (B), a point is shown for each subject. Horizontal bars denote the medians and whiskers denote the inter-quartile range.

Table 1. Frequency of solicited and unsolicited adverse events. Solicited reactogenicity was collected for 7 days after each vaccination. Each vaccine recipient is counted once at worst severity for any local and systemic parameter. Unsolicited AE percentages indicate the number of vaccine recipients with the given event (regardless of severity) divided by the total number who received PfSPZ Vaccine.

	$\leq 7.5 \times 10^3$ PfSPZ vac- cine/dose ($n =$ 9)	3×10^4 PfSPZ vaccine/dose ($n = 12$)	1.35×10^5 PfSPZ vac- cine/dose ($n =$ 19)	All groups (n $= 40$)
	No. (%)			
Any local reactogenicity				
None	8 (88.9)	10 (83.3)	12 (63.2)	30 (75.0)
Mild	1 (11.1)	1 (8.3)	7 (36.8)	9 (22.5)
Moderate	0 (0)	1 (8.3)	0 (0)	1 (2.5)
Severe	0 (0)	0 (0)	0 (0)	0 (0)
Any systemic reactogenicity				
None	6 (66.7)	7 (58.3)	6 (31.6)	19 (47.5)
Mild	3 (33.3)	4 (33.3)	9 (47.4)	16 (40.0)
Moderate	0 (0)	1 (8.3)	3 (15.8)	4 (10)
Severe	0 (0)	0 (0)	1 (5.3)	1 (2.5)
Unsolicited *adverse events				
Number with one or more adverse event	8 (88.9)	11 (91.7)	17 (89.5)	36 (90)
ALT and/or AST increased	5 (55.6)	6 (50)	5 (26.3)	16 (40)
Upper respiratory tract infection	3 (33.3)	2 (16.7)	5 (26.3)	10 (25.0)
Anemia	2 (22.2)	1 (8.3)	3 (15.8)	6 (15.0)
Leukopenia	2 (22.2)	0 (0)	2 (10.5)	4 (10)
Neutropenia	0 (0)	0 (0)	4 (21.1)	4 (10)
Gastroenteritis	0 (0)	1 (8.3)	3 (15.8)	4 (10)
Eosinophilia	1 (11.1)	1 (8.3)	1 (5.3)	3 (7.5)
Lymphopenia	0 (0)	1 (8.3)	2 (10.5)	3 (7.5)
Thrombocytopenia	0 (0)	2 (16.7)	1 (5.3)	3 (7.5)
Leukocytosis	0 (0)	0 (0)	2 (10.5)	2 (5.0)
Abdominal pain	0 (0)	0 (0)	2 (10.5)	2 (5.0)
Diarrhea	0 (0)	0 (0)	2 (10.5)	2 (5.0)
Dyspepsia	0 (0)	0 (0)	2 (10.5)	2 (5.0)
Muscle strain	0 (0)	1 (8.3)	1 (5.3)	2 (5.0)
Dizziness	0 (0)	1 (8.3)	0 (0)	1 (2.5)
Pruritus	0 (0)	1 (8.3)	0 (0)	1 (2.5)

*MedDRA terms for which one or more AE was assessed as possibly related to vaccination are shown; adverse event terms for which no events were considered related are not shown. Frequency of any hepatic enzyme adverse event (alanine aminotransferase (ALT) increased, aspartate aminotransferase (AST) increase, or both) are counted together; see Table S4 for more detail on these events.