Journal of Infectious Diseases Advance Access published June 15, 2016

Safety and High Level Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01$_B$ with ChAd-MVA Vectored Vaccines Expressing ME-TRAP

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Abstract

Background: The need for a highly efficacious vaccine against *Plasmodium falciparum* remains pressing. In this controlled human malaria infection (CHMI) study, we assessed the safety, efficacy and immunogenicity of a schedule combining two distinct vaccine types in a staggered immunization regimen: one inducing high-titer antibodies to CSP (RTS,S/AS01$_B$) and the other inducing potent T-cell responses to TRAP using viral vectors.

Method: 37 healthy malaria-naïve adults were vaccinated with either ChAd63-MVA expressing ME-TRAP and 3 doses of RTS,S/AS01$_B$ (Group 1, n=20) or 3 doses of RTS,S/AS01$_B$ alone (Group 2, n=17). CHMI was delivered by mosquito bites in 33 vaccinated subjects at week 12 after first vaccination, and 6 unvaccinated controls.

Results: No SUSAR or SAEs related to vaccination were reported. Protective vaccine efficacy was observed in 14/17 (82.4%) subjects in Group 1 and 12/16 (75%) subjects in Group 2. All control subjects were diagnosed with blood stage malaria. Both vaccination regimens were immunogenic. 14 protected subjects underwent repeat CHMI 6 months after initial CHMI; 7/8 (87.5%) Group 1 subjects and 5/6 (83.3%) Group 2 subjects remained protected.

Conclusion: The high level of sterile efficacy observed in this trial is encouraging for further evaluation of combination approaches using these vaccine types.

Clinicaltrials.gov Registration: NCT01883609

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Introduction

Malaria remains one of the leading causes of mortality globally,[1] and there is urgent need for a vaccine. The majority of deaths are in children less than 5 years old, with this age group accounting for approximately 306,000 deaths in 2015. The enormous economic and social consequences of malaria have been well documented.[2] Efforts to develop effective vaccines are complicated by the complex immunology of malaria infection, and no reliable natural model of complete immunity exists. Despite this, a small number of candidate vaccines have shown partial efficacy against experimental and natural human infection with the current leading vaccine being the recombinant protein in adjuvant, RTS,S/AS01. RTS,S targets the circumsporozoite protein (CS), which is expressed by the *P. falciparum* sporozoite at the pre-erythrocytic stage, and was the first subunit vaccine to show high rates of sterile efficacy, typically 50%, in controlled human malaria infection (CHMI) studies[3]. In a large African phase III trial, this vaccine has shown efficacy against clinical malaria of 55·8% (97·5% CI 50·6–60·4) in children aged 5–17 months and 31·3% (23·6–38·3) in infants aged 6–12 weeks over the first year after vaccination.[4, 5] Vaccine efficacy wanes over time, but can be enhanced by a fourth dose.[6] Analysis of the immunological correlates of efficacy of this vaccine suggest vaccine-induced antibodies targeting CS are the most important mediators of RTS,S-induced protection against malaria,[3] although no antibody level threshold has been shown to be predictive of efficacy. The rate at which anti-CS antibodies wane is similar to the rate at which efficacy declines[7, 8] suggesting that anti-CS antibodies may also be associated with the duration of protection. A number of factors,

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including age at vaccination, HIV status and high baseline anti-CS antibody titers impact anti-CS antibody titers after vaccination with RTS,S.[9]

The pre-erythrocytic stage of *P. falciparum* infection presents an attractive target for an efficacious human vaccine, as sufficient reduction in the number of viable merozoites reaching the blood from the liver will prevent parasitization of red blood cells and initiation of the symptomatic blood stage of infection. Anti-CS antibodies can target sporozoites for destruction prior to hepatocyte invasion. As sporozoites travel from the skin to liver within minutes, it may be difficult for a vaccine to achieve complete protection against *P. falciparum* based solely on antibodies to sporozoites. The liver stage of infection provides a longer window of opportunity for cell-mediated immunity to recognize and destroy infected hepatocytes. ChAd63 ME-TRAP and MVA ME-TRAP are viral vectored vaccines, and when administered in a prime-boost sequence at an eight-week interval is a leading candidate vaccine strategy targeting the liver stage of infection.[10] The chimpanzee adenovirus (ChAd63) and modified vaccinia Ankara (MVA) viral vectors deliver the recombinant ME-TRAP insert, which generates a potent cellular immune response against the liver-stage *P. falciparum* antigen, thrombospondin related adhesion protein (TRAP), of greater magnitude than the cellular response induced by RTS,S/AS01. This strategy showed durable partial efficacy in two Phase IIa sporozoite challenge trials in the UK[11, 12] using the 3D7 parasite as a challenge strain. The viral vector encoded *P. falciparum* TRAP allele is from the heterologous T9/96 strain and induced T cell responses correlate with efficacy.[11] Therefore these are effectively heterologous strain CHMI studies. Interestingly, a higher level of efficacy of 67% [95% CI 33-83] against *P. falciparum* infections detected by PCR was observed in a

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Phase IIb trial in Kenyan adults.[13] Again, T cells to TRAP peptides correlated with vaccine efficacy but the short duration of malaria transmission and follow-up at this trial site precluded analysis of the durability of vaccine-induced protection.[13] This heterologous prime-boost strategy showed potent cellular immunogenicity in adults in the UK,[11] as well as adults and infants in malaria endemic areas,[13-15] (Ewer et al submitted) and has an excellent track record of safety and tolerability in these populations. Analysis of the potential utility of combining anti-sporozoite and anti-liver-stage vaccines have suggested a likely additive or synergistic effect[16] in keeping with findings from pre-clinical studies.[17, 18]

In this phase I/IIa, open-label, CHMI study, we assessed the safety, immunogenicity and efficacy of a vaccine schedule combining these two distinct candidate vaccine types in a staggered immunization regimen: one that induces very high titer antibodies to CS using RTS,S/AS01$_B$ and the other inducing potent T cell responses to TRAP using viral vectors.

Methods

Participants

Recruitment and vaccination was conducted at three UK sites, in Oxford, Southampton and London. The CHMI procedure was performed as previously described[19] using five infectious bites from *P. falciparum* 3D7-strain infected *Anopheles stephensi* mosquitoes at Imperial College, London. All subjects were infected with a single batch of mosquitoes at the initial CHMI, and with a second single batch at the repeat CHMI. Infected mosquitoes were supplied by the Department of Entomology, Walter Reed Army Institute of Research, Washington

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DC, USA. Healthy, malaria-naïve males and non-pregnant females aged 18-45 years were invited to participate in the study. All volunteers gave written informed consent prior to participation, and the study was conducted according to the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice (GCP).

Ethical and Regulatory Approval

Necessary approvals for the study were granted by the UK National Research Ethics Service, Committee South Central – Oxford A (Ref: 13/SC/0208), the Western Institution Review Board (Ref: 20130698) and the UK Medicines and Healthcare products Regulatory Agency (Ref: 21584/0317/001-0001). The trial was registered with ClinicalTrials.gov (Ref: NCT01883609). The Local Safety Committee provided safety oversight and GCP compliance was independently monitored externally by the Clinical Trials and Research Governance Team (CTRG) of the University of Oxford.

Study Design

This Phase IIa, open-label partially randomized challenge trial consisted of 4 cohorts. Allocation to study group occurred at screening based on subject preference. Any subjects without a preference were randomized to vaccine Group 1 or 2. Group 1 (n=20) received 5 vaccinations (RTS,S/AS01$_B$ 50 µg at 0, 4 and 8 weeks, ChAd63 ME-TRAP 5 x 10^{10} virus particles (vp) at 2 weeks, and MVA ME-TRAP 2 x 10^8 plaque forming units (pfu) at 10 weeks); Group 2 (n=20) received 3 vaccinations (RTS,S/AS01$_B$ 50 µg at 0, 4 and 8 weeks); Group 3 (n=6) received no vaccinations. All vaccinations were administered intramuscularly (IM) into the deltoid region of the

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arm. In each volunteer, all RTS,S/AS01$_B$ injections were given in one arm, and all viral vector injections were given in the contralateral arm. All subjects underwent initial CHMI by mosquito bite at the same time (week 12 after first vaccination for vaccinated subjects). Following CHMI, a diagnosis of blood stage malaria infection was made in subjects with symptoms suggestive of malaria and positive thick film microscopy, or qPCR result >500 parasites/ml if either thick film was negative, or symptoms were absent. [12] Vaccinated subjects who had not developed blood stage malaria by day 21 after CHMI were deemed to exhibit sterile protection and were invited to undergo repeat CHMI 6 months later, for which an additional control group was recruited (Group 4).

Further details of the study sites, inclusion/exclusion criteria, the vaccines, clinical follow-up, safety monitoring, malaria treatment and diagnosis, immunological and molecular methods, and statistics can be found in the supplementary material.

Results

Participants

Eighty subjects were screened for eligibility and 48 subjects were identified as eligible. Twenty subjects were allocated to group 1 to receive RTS,S/AS01$_B$ and viral vectors encoding ME-TRAP. Seventeen subjects were allocated to group 2 to receive RTS,S/AS01$_B$ only. Six unvaccinated controls were recruited to group 3 for the initial CHMI, and 5 subjects were allocated to group 4 for the repeat CHMI. Vaccinations took place between 2nd September 2013 and 13th November 2013. Prior to CHMI, 3 subjects withdrew from group 1, and 1 subject withdrew from Group 2. There were no withdrawals due to safety concerns and no pre-defined study stopping or holding

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rules were activated. CHMI was performed on the 25th and 26th of November 2013, and repeat CHMI on 13th May 2014 (Figure 1).

Protective efficacy against CHMI

A total of 39 subjects participated in the initial CHMI (17 subjects from Group 1, 16 subjects from Group 2 and 6 subjects in Group 3),which was conducted over 2 days. Three subjects in Group 1 and 4 subjects in Group 2 were diagnosed with malaria before day 21 post-challenge, resulting in a sterile efficacy of 82.4% [95% CI 64-100] and 75% [95% CI 54-96] respectively (**Figure 2**). Median time to diagnosis was 14.5 days in Group 1 and 13.25 days in Group 2. All 6 control subjects were diagnosed with malaria with a median time to diagnosis of 12.25 days (range 11-13, SD 0.7 days). Both vaccine regimens demonstrated significantly reduced risk of malaria infection over controls in the per protocol analysis (Group 1 hazard ratio (HR) 0.065, p <0.0001; Group 2 HR 0.12, p <0.0001 for Group 2), but there was no significant difference in efficacy between vaccine regimen (HR 0.65, p 0.57). Eight Group 1 and 6 Group 2 protected subjects agreed to undergo repeat CHMI. A single subject in each of Groups 1 and 2 were diagnosed with malaria at day 17 and day 14.5 respectively, and all 5 control subjects developed malaria with a mean time to diagnosis of 12.4 days (Median 12.5, range 11.5-13.5, SD 0.8).

Safety

The safety profile of a 3 dose regimen of RTS,S/AS01$_B$ and of ChAd63-MVA ME-TRAP when given separately to malaria-naïve adults has been described previously,[3, 10-12, 20] and a similar reactogenicity profile was observed after vaccination in this

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study. The majority of adverse events (AEs) following vaccinations in both Group 1 and Group 2 were mild in severity and self-limiting. There were no serious adverse events (SAEs) related to vaccination, and no Suspected, Unexpected Serious Adverse Reactions (SUSAR)s. Solicited and unsolicited AEs following vaccination are detailed in supplementary tables S1-S12.

Humoral response to vaccination

Anti-TRAP IgG antibodies (Abs) were measured in Group 1 subjects only (Figure 3), and geometric mean titers (GMTs) peaked on the day before challenge (C-1) at 947 Elisa Units (EU) [95% CI 617-1455]. No association was detected between anti-TRAP IgG levels and efficacy (Spearman's r=-0.25, p=0.3). Anti-CS Abs were measured at key time points in all vaccinated subjects. Serum anti-CS Abs peaked at C-1 in both vaccinated groups with peak GMT of 1733 EU [95% CI 1240-2422] and 1824 EU [95% CI 1330-2502] in groups 1 and 2 respectively. There was no significant difference in anti-CS Ab GMTs between group 1 and group 2 at C-1 (Mann-Whitney p>0.99). Anti-CS Ab GMTs at C-1 were significantly higher in protected subjects than non-protected subjects (Figure 3), (protected GMT 1985 EU [95% CI 1584-2487]; non-protected GMT 1177 [95% CI 627-2209]; Mann-Whitney p=0.035). There was a correlation between Anti-CS Ab titer and parasite density at day 7.5, (Spearmans r=-0.4, p=0.018). There was no significant difference in avidity of anti-CS Abs between protected and non-protected volunteers at any timepoint, however avidity significantly increased between Day 28 and C-1 in protected but not non-protected volunteers (p=0.001 and p>0.99 respectively, Wilcoxon matched-pairs test). Avidity also increased between Day 56 and C-1 in protected but not non-protected

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volunteers (p<0.0001 and p=0.375 respectively, Wilcoxon matched-pairs test). In the protected vaccinated subjects who underwent repeat CHMI, avidity on the day before re-challenge (RC-1) remained significantly higher than at D28 (P=0.002, Mann-Whitney test).

Cellular response to vaccination

T-cell responses against ME-TRAP were measured in all Group 1 subjects by ex-vivo interferon-gamma (IFNγ) ELISPOT (Figure 4). Peak responses after ChAd63 ME-TRAP vaccination were detected 21 days later; GM 539 spot forming cells (SFC) per million peripheral blood mononuclear cells (PBMC) [95% CI 300-968]. Peak responses after MVA ME-TRAP vaccination were detected 7 days later; median 1520 SFC [inter-quartile range (IQR) 699-3305]. T-cell responses against ME-TRAP were well maintained over time with a median of 464 SFC [IQR 231-933] 90 days after initial challenge, and 342 SFC [IQR 143-815] in participating subjects the day before repeat CHMI (RC-1).

T-Cell responses against CS were measured in all vaccinated subjects by IFNγ ELISPOT (Figure 4). Responses peaked in Group 1 at C-1 (4 weeks after final dose of RTS,S/AS01$_B$); median of 36 SFC [IQR 12-176]with responses at 12 SFC [IQR 12-70] in Group 2 at the same time point . No association between ELISPOT responses to TRAP or CS and vaccine efficacy was detected (Spearmans r=-0.01, p=0.98 and r=-0.001, p=0.0996 for TRAP and CS respectively).

Flow cytometry using Intracellular Cytokine Staining (ICS) was performed for CS and HBsAg at day 42 after first vaccination and C-1 using cryopreserved PBMC. In this assay, responses were described as the number of cells per million $CD4^+$ or $CD8^+$ T

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cells expressing at least two markers from CD154 (CD40 ligand), IFNγ, IL-2 and TNFα (Figure 5A and B). CS-specific $CD4^+$ T cell responses peaked at day 42 (two weeks after the second dose of RTS,S) in both groups and no significant differences were detected between Group 1 and 2 either at day 42 or C-1 (Figure 5C). CS-specific $CD8^+$ T cell responses were not detected at any significant frequency. A positive association was detected between the number of polyfunctional $CD4^+$ T cells at D42 and the level of anti-CS IgG in serum at C-1 (Figure 5D, Spearman's r=0.4, p=0.03). Vaccination with RTS,S increased the frequency of HBS-specific $CD4^+$ polyfunctional T cells in both groups at all time points after vaccination (Figure 5E and F).

Flow cytometry was also performed on freshly isolated PBMC using CS peptides (Group 1 and 2 subjects) and ME-TRAP peptides (Group 1 subjects only) at C-1. Group 1 responses to TRAP T9/96 and 3D7 were comparable across all cytokines and CD107a (Kruskall-Wallis test with Dunn's Correction P<0.0001), with all volunteers exhibiting at least one positive cytokine response to both TRAP strains (Figure 6A and B). A positive response was measured to CS in 15/17 (83%) group 1 volunteers compared to just 9/16 (56%) volunteers in group 2, with a significantly higher frequency of IFNγ-producing CD4+ T cells in group 1 (Figure 6C).

By ex-vivo IFNγ ELISpot to CS peptides, a trend was observed towards higher responses in Group 1 compared to Group 2 (Mann-Whitney test on combined groups p=0.0517); when assessed by peptide pool, a significant trend to higher responses was observed in pool 1 (Mann-Whitney test p=0.0380). This is likely due to CS epitope(s) present in the Multi-Epitope string of ChAd63 ME-TRAP and MVA ME-TRAP. Ex-vivo IFNγ data suggests that this epitope lies towards the N terminus of CS, as identified by a significantly higher Group 1 response to peptide pool 1. The

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Multi Epitope string contains 2 epitopes present in pool 1: CD8 epitope cp26 KPKDELDY; and CD4 epitope DPNANPN, as part of longer ME sequence DPNANPNNVDPNANPNV (Table 1). As the main differences in ICS IFNγ production were in the CD4+ T cell compartment, epitope DPNANPN could be responsible for the enhanced CS responses in group 1. This epitope is not present in RTS,S, so is solely induced by ChAd63.MVA ME-TRAP prime boost vaccination.

Discussion

Both RTS,S/AS01$_B$ and ChAd63-MVA encoding ME-TRAP have previously demonstrated partial efficacy in CHMI trials[3, 11, 12, 20] but this is the first study in which RTS,S/AS01$_B$ and ChAd63-MVA ME-TRAP have been given to subjects in the same vaccine regimen. In this study we have shown that administering these vaccines sequentially is safe, with no SUSARs, and no vaccine related SAEs. The reactogenicity profile observed in the subjects who received the combined vaccine regimen (Group 1) was similar to that observed when RTS,S/AS01$_B$ or ChAd63-MVA ME-TRAP were given alone in a malaria-naïve adult population. [3, 11, 12, 20]

Furthermore, we have demonstrated that these vaccine candidates remain immunogenic when the regimens are combined. Anti-CS antibodies were not significantly different between Group 1 and Group 2 at C-1, and peak numbers of TRAP-specific T-Cells in Group 1 were similar to those observed with ChAd63-MVA ME-TRAP administered alone in a previous study.[11] Geometric mean titers of anti-CS antibodies were significantly higher in protected subjects at C-1, but there was no

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correlation between any TRAP or CS-specific T cell assays or TRAP-specific IgG and protection.

In this study we observed a high level of protective efficacy in both vaccine arms. A higher proportion of subjects in Group 1 remained protected following CHMI than in Group 2 (82.4% vs. 75% respectively), though this difference was not statistically significant (p=0.57). This high level of vaccine efficacy was also seen to be durable at 6 months with 87.5% and 83.3% of initially protected subjects who underwent repeat CHMI remaining protected in Groups 1 and 2 respectively. In addition, a higher proportion of subjects in Group 1 reached the secondary efficacy endpoints of delay to malaria diagnosis and delay to PCR parasitemia compared with Group 2. The trends observed in this study for initial challenge, rechallenge and effects on the pre-patent period are encouraging for further evaluation of the Group 1 regimen, but the numbers in this study are small, and the differences observed not statistically significant. In 2013, a CHMI study of the cryopreserved whole sporozoite (PfSPZ) vaccine reported sterile efficacy of 100% in the high dose regimen consisting of 5 doses of 1.35 x 10^5 parasites.[21] However, the vaccinee numbers in the high dose group were small (n=6) and only 5/6 (83.3%) of the unvaccinated controls developed blood stage infection, raising concerns over the infectivity of the parasites used in that CHMI. The results observed in the trial we present in this article, therefore appear to represent the highest published sterile vaccine efficacy in any CHMI study in which all the control subjects were infected.

The level of protective efficacy observed in the RTS,S/AS01$_B$ alone group (75%) is higher than has been reported in most prior CHMI studies of this vaccine regimen.[3,

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20]. The mean time to patency in the control group of 12.2 days indicates that this was not an unusually weak challenge, and the vaccination and CHMI methodology used in this trial are largely comparable to other CHMI studies of this dosing schedule of RTS,S/AS01.[3] Practical limitations on study size are a factor for both this study and prior CHMI studies of RTS,S, resulting in a relatively small historical dataset. In light of this, it is possible that the higher efficacy seen in the RTS,S alone group in this trial is a chance finding, due in part to small numbers, or that further CHMI studies of RTS,S/AS01 in malaria-naïve subjects, including further evaluation of differing dosing regimens and schedules would further clarify the efficacy of the vaccine in this setting. The study was designed to have 84% power to detect a significant ($p < 0.05$) increase in sterile efficacy in Group 1 to 90%, and with 69% power to detect a significant increase to 85% compared to Group 2. This power calculation assumed an expected 50% sterile efficacy in Group 2.[3] The increase in efficacy to 82.4% in Group 1 from 75% in Group 2 observed in this trial was not statistically significant ($p=0.69$), but power to detect a statistically significant improvement was very limited. Practical limitations of CHMI trials makes conducting large studies difficult, and designing future studies with sufficient power would be complicated, assuming efficacy of 75% in an RTS,S/AS01$_B$ alone group. One alternative approach is to wait longer post immunization to allow vaccine efficacy to wane and thereby provide greater power to detect additive or synergist effects of combination vaccines. Further consideration of this issue, and of the practical limitations of CHMI studies with challenge at 3 to 4 weeks post last vaccination in future trial designs is warranted.

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We undertook a re-challenge of protected subjects at 6 months after the initial CHMI, 7/8 (87.5%) Group 1 subjects and 5/6 (83.3%) Group 2 subjects remained protected. By simply calculating the product of the percentage efficacies in the two CHMIs one can estimate vaccine efficacy at six to seven months after the immunizations as a measure of durable sterile protection. For Group 1 this is 72% (14/17 x 7/8 x 100%) and for Group 2, 62.5% (12/16 x 5/6 x 100%). Again the durable protection rate at this time point in Group 2 appears higher than in previous re-challenge trials with RTS,S/AS01$_B$ administered three times[3] and the Group 1 protection rate is even higher. This durability at six months is also encouraging for continued investigation of combination vaccine approaches and supports the consideration of delayed CHMI as an approach to evaluating improvements in efficacy provided by vaccines that confer substantial short term efficacy.

In this trial, we present data from a combined vaccine regimen in which subjects received a total of five vaccinations over a 10-week period. A priority for future studies should be to evaluate the effect of simplifying the vaccination schedule. A study evaluating the concomitant administration of RTS,S with viral vectors expressing ME-TRAP, thereby reducing the total number of vaccinations in a more practical schedule for potential deployment is currently underway.

These results are encouraging for further evaluation of malaria vaccine regimens that combine viral vectors with protein subunits, and also vaccine regimens that target multiple stages of the malaria parasite life cycle.

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Footnotes

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Acknowledgements

We thank M. Smith, R. Lopez-Ramon and A. Cook for clinical assistance; N. Lella and S. Pawluk for logistical support; J. Furze for laboratory assistance; the staff at the NIHR Wellcome Trust Clinical Research Facilities at the Southampton and Hammersmith Hospital sites; S. Davidson and colleagues at the WRAIR Entomology laboratories for supply of infected mosquitoes for CHMI; WRAIR Malaria Serology Laboratory (MSL) for additional serology; B. Angus for local safety oversight; the study volunteers; Philippe Moris at GSK for CMI testing.

Conflict of Interest

A. V. S. H. and S. C. G. are named inventors on patent applications covering malaria vectored vaccines and immunization regimens. D. M, M. L and R. W. B are employees of GSK, which is developing vectored vaccines for malaria and other diseases. S.N.F. acts on behalf of the University of Southampton/University Hospital Southampton NHS Foundation trust as chief and principal investigator for clinical trials Sponsored by vaccine manufacturers including GSK but receives no personal payments for the work. All other authors report no potential conflicts.

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Funding Statement

This work was funded primarily by the PATH Malaria Vaccine Initiative (MVI); in addition, the work was supported by the United Kingdom National Institute of Health Research (NIHR) infrastructure, through the NIHR Oxford Biomedical Research Centre, the Southampton NIHR Wellcome Trust Clinical Research Facility, and the Imperial College NIHR Wellcome Trust Clinical Research Facility; This article/paper/report presents independent research funded by PATH MVI and supported by the NIHR CRF and BRC at Imperial College Healthcare NHS Trust. The views expressed are those of the author(s) and not necessarily those of MVI, the NHS, the NIHR or the Department of Health.

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FIGURE LEGENDS

Figure 1: Flow of study design and volunteer recruitment. Twenty-seven subjects were excluded according to inclusion/exclusion criteria. Three subjects withdrew consent after screening, but before enrolment. Two subjects were deemed eligible as control subjects but only after Group 3 enrolment was complete. They were kept as backup subject in case of last minute withdrawals from Group 3, but never underwent CHMI. 17 subjects expressed a preference as to which vaccine group to be allocated to, and were assigned accordingly. 20 subjects expressed no preference for vaccine group allocation, and were therefore randomized to group by the study statistician. ChAd63, Chimpanzee adenovirus serotype 63; MVA, Modified vaccinia virus Ankara; ME-TRAP, multiple-epitope thrombospondin-related adhesion protein; CHMI, Controlled human malaria infection.

Figure 2: Efficacy of RTS,S/AS01$_B$ with ChAd63-MVA ME-TRAP immunization and RTS,S/AS01$_B$ immunization alone following *Plasmodium falciparum* 3D7 sporozoite challenge. Kaplan–Meier survival analyses. Log-rank test for significance. A, Kaplan–Meier survival analysis of time to treatment following initial CHMI. Mean time to diagnosis was 12.2 (± 0.7) days for unvaccinated controls. Seventeen out of 17 subjects in Group 1 (100%) and 14 out of 16 subjects in Group 2 (87.5%) were undiagnosed by day 21, or diagnosed after the control mean +2 SD. B, Kaplan–Meier survival analysis of time to first sample with >20 parasites/mL detected by quantitative polymerase chain reaction (qPCR). Mean time to endpoint was 7.4 (± 0.7) days for unvaccinated controls. Sixteen out of 17 subjects in Group 1 (94.1%)

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and 15 out of 16 subjects in Group 2 (93.8%) did not reach this endpoint, or did so after the control mean +2 SD. C, Kaplan–Meier survival analysis of time to first sample with >500 parasites/mL detected by qPCR. Mean time to endpoint was 9.8 (±0.8) days for unvaccinated controls. Seventeen out of 17 subjects in Group 1 (100%) and 15 out of 16 subjects in Group 2 (93.8%) did not reach this endpoint, or did so after the control mean +2 SD. D, Kaplan–Meier survival analysis of time to treatment following repeat CHMI in protected subjects. Abbreviations: CHMI, controlled human malaria infection; controls, unvaccinated volunteers undergoing CHMI; ME-TRAP, multiple epitope–thrombospondin related adhesion protein.

Figure 3: Antibody responses to vaccination measured by enzyme-linked immunosorbent assay (ELISA). A, Anti-thrombospondin adhesion protein (TRAP) immunoglobulin G (IgG) antibody responses after vaccination with RTS,S/AS01$_B$ plus ChAd63-MVA ME-TRAP (Group 1 subjects only). Lines represent group medians. B, Anti-circumsporozoite protein (CS) immunoglobulin G (IgG) antibody responses after vaccination with RTS,S/AS01$_B$ plus ChAd63-MVA ME-TRAP (Group 1; blue) or RTS,S alone (Group 2; black). Line represents group median. C, Comparison of Anti-CS IgG antibody responses between Group 1 (Blue) and Group 2 (Black) as measured on the day before CHMI (C-1). No significant difference (NSD) P>0.999 by Mann-Whitney analysis. Comparison of anti-CS IgG antibody responses in volunteers that were sterilely protected or not. Lines represent geometric mean. D, Correlation between anti-CS IgG titres at C-1 and parasite density at day 7 post-challenge. Spearman's r=-0.4, p=0.018. E, Avidity of total IgG against the NANP repeat region of CSP. Significant increase in avidity between D28 and C-1 in protected, but not non-

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protected volunteers. P=0.001, Wilcoxon matched-pairs test. Avidity of total IgG remained significantly higher at time of 2nd CHMI (RC-1), compared to D28. P=0.002, Mann-Whitney test. Lines represent geometric mean. Abbreviations: ChAd63, simian adenovirus 63; CHMI, controlled human malaria infection; EU, ELISA units; MVA, modified vaccinia virus Ankara; C+, value denotes elapsed time after CHMI in days.

Figure 4: Antigen-specific T-cell responses to vaccination measured by IFN γ ELISPOT. A, Median T-cell responses to ME-TRAP. B, Median T cell responses to CSP peptide pools are shown for Group 1 (RTS,S/AS01 and ME-TRAP, blue line) and Group 2 (RTS,S/AS01, black line).

Figure 5: T cell responses by flow cytometry on cryopreserved peripheral blood mononuclear cells pre- and post-vaccination for CSP and Hepatitis B surface antigen (HBS). Polypositive indicates number of cells per million expressing 2 or more of the following markers; CD154 (CD40 ligand), IFN γ , Il-2 and TNF α . A and B, Number of CSP-specific polypositive CD4+ or CD8+ T cells per million in Group 1 and 2 respectively. C, Comparison of CD4+ polypositive T cells at peak time point post vaccination (day 42) and day before CHMI (C-1) for Group 1 (G1) and 2 (G2). D, Correlation between peak CSP-specific CD4+ polypositive frequency and anti-CS IgG level at C-1 (r=0.4, p=0.03, Spearman's test). E and F, Number of HBS-specific polypositive CD4+ or CD8+ T cells per million in Group 1 and 2 respectively.

Figure 6: Intracellular cytokine staining of PBMC one day prior to CHMI (27 days post final RTS,S vaccination and 13 days post MVA ME-TRAP vaccination), showing

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CD107a expression frequency and frequencies of cytokine-secreting cells, as a percentage frequency of parent CD4+ and CD8+ T cells. Geometric mean of each response is shown in response to stimulation with A) TRAP T9/96 peptides (homologous to vaccine insert) by Group 1, B) TRAP 3D7 peptides (homologous to CHMI challenge strain) by Group 1 and C) CS peptides by Group 1 and 2. D) Ex-vivo IFN-γ ELISpot responses of Group 1 and 2 volunteers to CS peptides split into 3 peptide pools and a combined pool, with background subtracted. Dotted line shows the median background ELISpot response, setting the positive response threshold. N=17 for group 1 and n=16 for group 2. Data points represent individual volunteers.

Table 1: Comparison of peptide sequences present in Multi-Epitope string of ME-TRAP and the T cell region of RTS,S (Lalvani et al 1999).

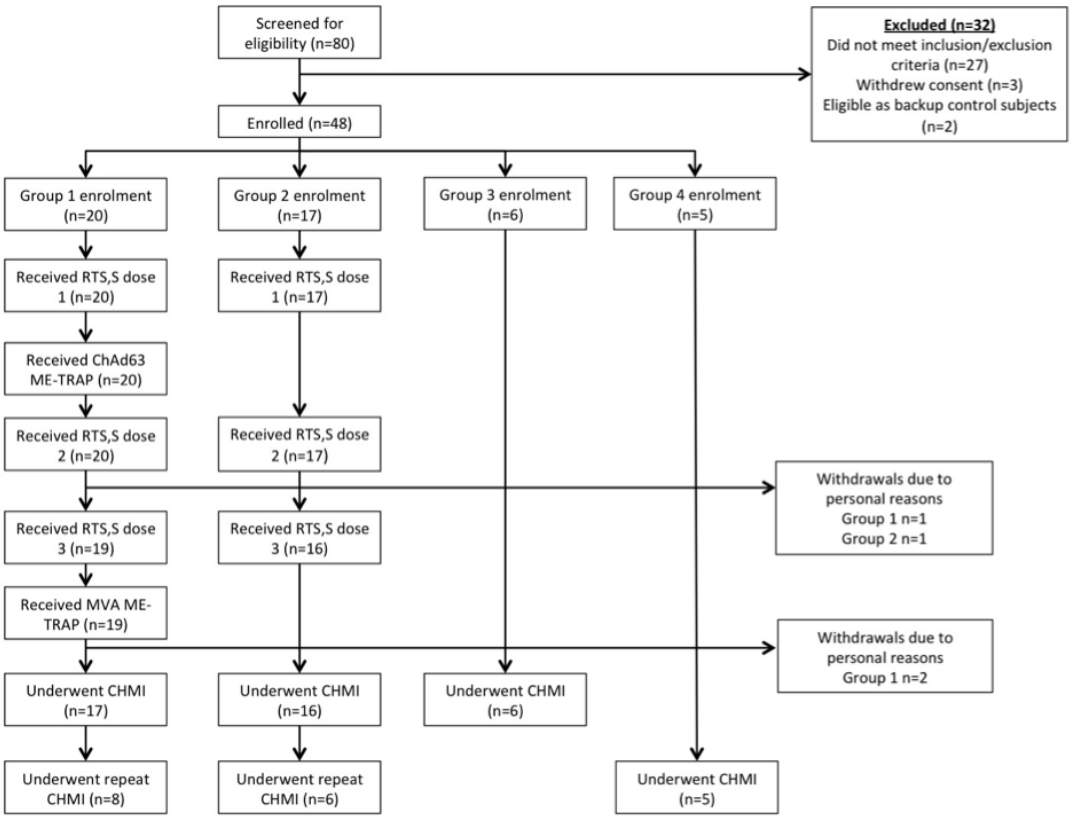
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Epitope Sequence	CS Amino Acid Position	Epitope Type	Present in ME string	Present in RTS,S	Present in ELISpot CS Peptides	ELISpot Pool Number
DPNANPNVDP NANPNV	111-126 (16)	CD4	Yes	No	Yes, DPNANPN only	1
NMPNDPN RNV	286-293 (8)	CD8	Yes	Yes	Yes, PNDPN RNV only	1
YL NKIQNSL	319-327 (9)	CD8	Yes	Yes	Yes, full length	2
KPKDELDY	353-360 (8)	CD8	Yes	Yes	Yes, full length	3

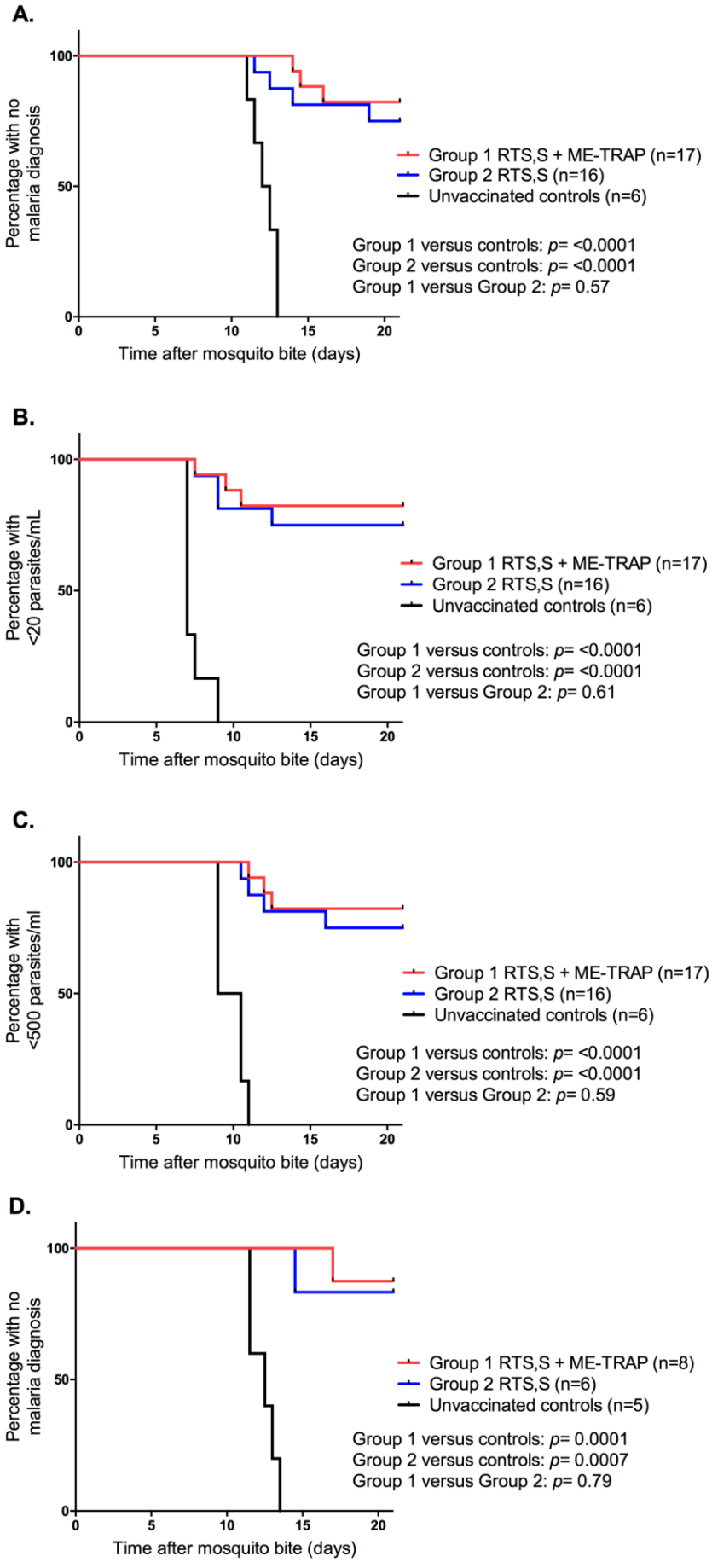
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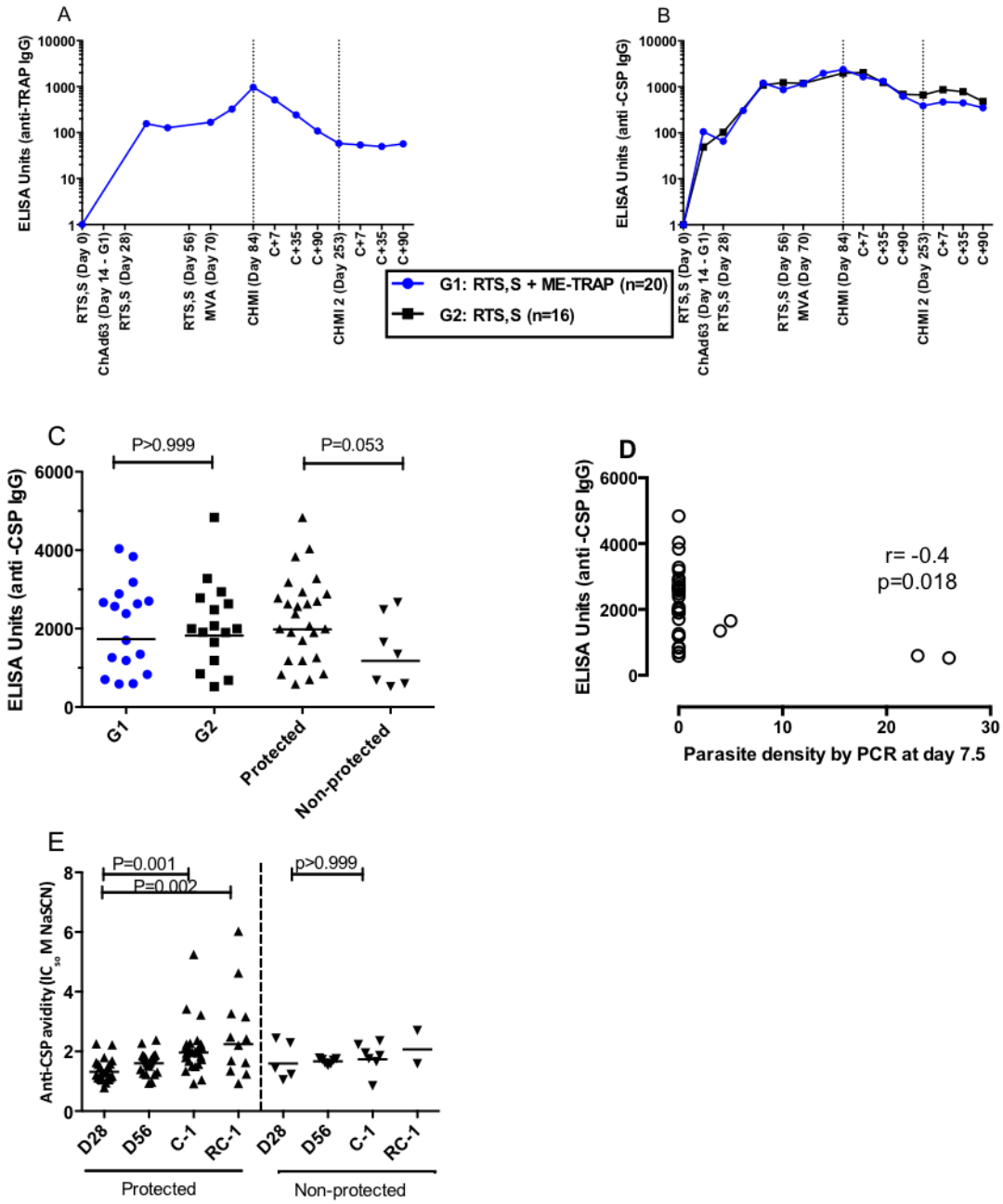


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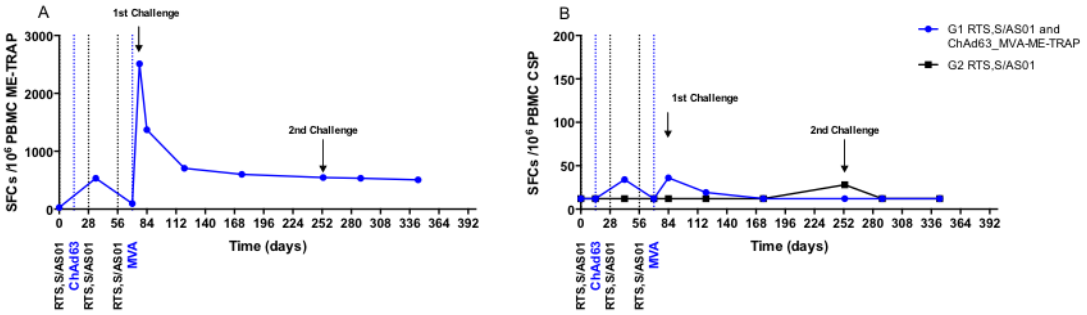


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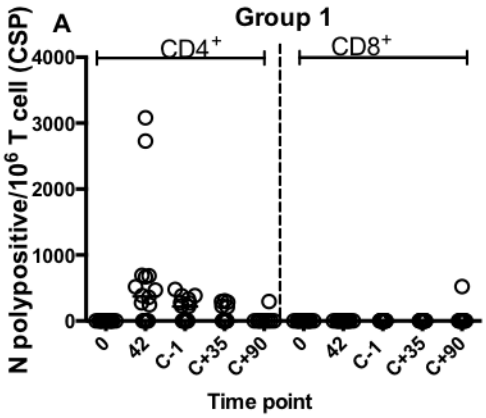
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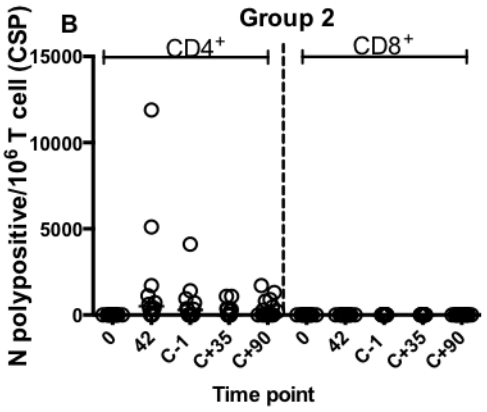
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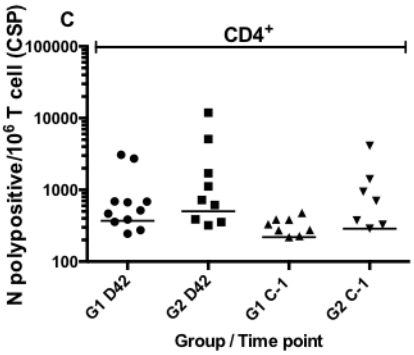


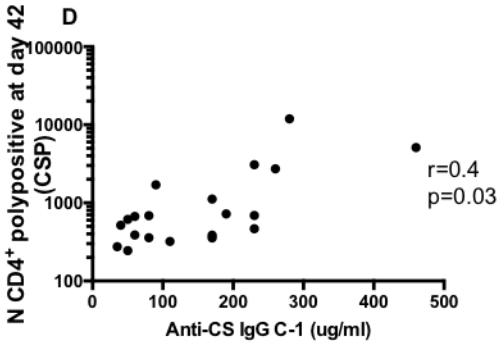
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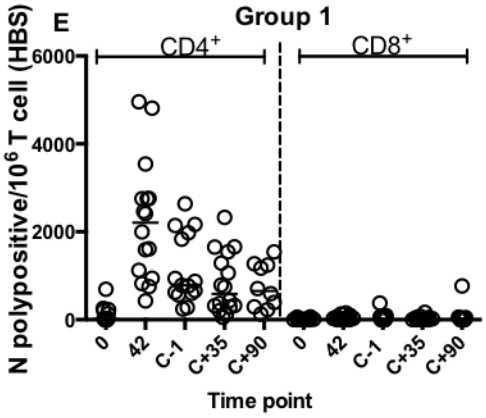
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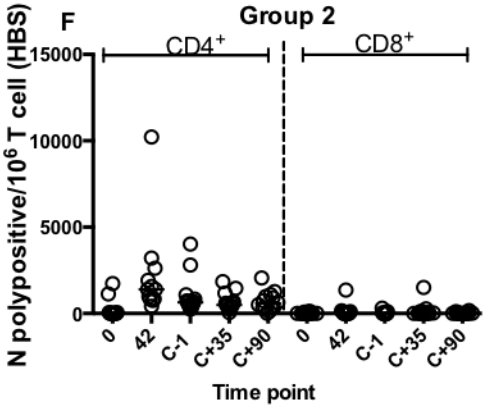






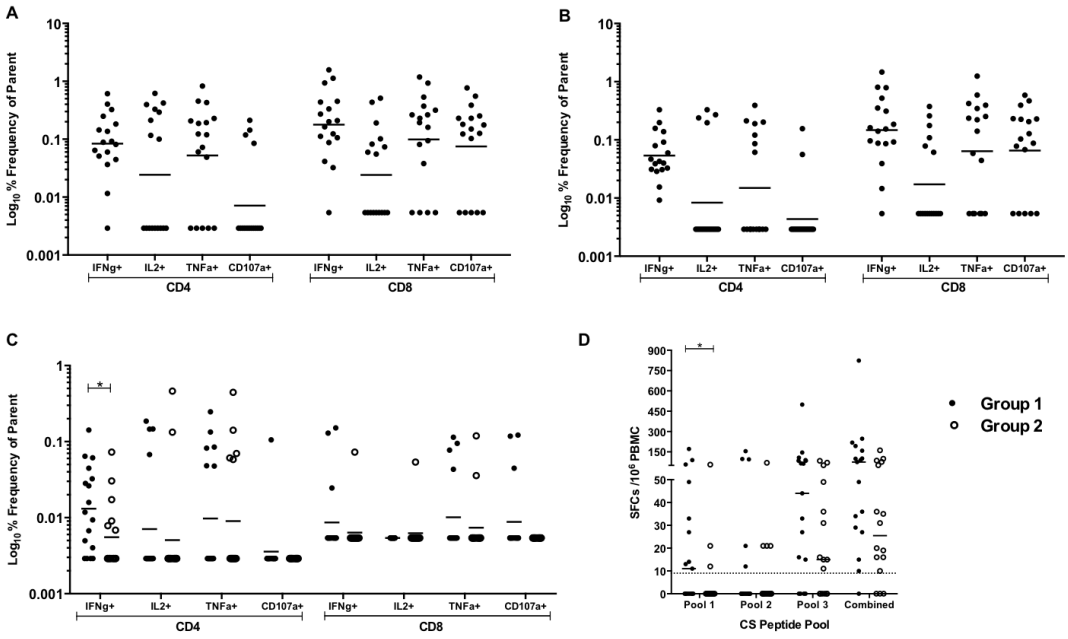






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