RCSI

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Final Report to the EPA on of VAC038, a malaria vaccine study.

SNIF No: B/IE/11/451(a) and B/IE/11/451(b)
GMO register number: G0451-01

Title

A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS administered alone and with MVA CS.

Registration:

ClinicalTrials.gov (Ref: NCT01450280);
http://clinicaltrials.gov/ct2/show/NCT01450280?term=ChAd63+cs&rank=1

Results of the clinical trial

Abstract:

Methodology: We conducted a Phase Ia, non-randomized clinical trial in 24 healthy, malaria-naïve adults of the chimpanzee adenovirus 63 (ChAd63) and modified vaccinia virus Ankara (MVA) replication-deficient viral vectored vaccines encoding the circumsporozoite protein (CS) of *P. falciparum*.

Results: ChAd63-MVA CS administered in a heterologous prime-boost regime was shown to be safe and immunogenic, inducing high-level T cell responses to CSP (median 1523.75, mean 1947 SFU/million PBMC) for ChAd63 CS dose $5x10^9$ vp; (median 1048.75, mean 1659 SFU/million PBMC) for ChAd63 CS dose $5x10^{10}$ vp, with a mixed CD4+ / CD8+ phenotype. Serum IgG responses to CS were modest (median Day 14 antibody titre 350), but persisted throughout late follow up (day 140 median antibody titre 187.).

Conclusions: ChAd63-MVA is a safe and highly immunogenic delivery platform for CS antigen in humans which warrants further efficacy testing. ChAd63-MVA is a promising heterologous prime-boost vaccine strategy that could be applied to numerous other diseases where strong cellular and humoral immune responses are required for protection.

Participants

The study was conducted at the Clinical Research Centre, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland. Healthy, malaria-naïve males and non-pregnant females aged 18-50 were invited to participate in the study. Allocation to study groups (Figure 1) occurred at screening based on volunteers' availability. Twelve volunteers were vaccinated intramuscularly (IM) with 5×10^9 viral particles vp ChAd63 CS (in 300µL of 0.9%

NaCl and administered in 350µL) (groups 1A & 1B). Eight of these volunteers were subsequently vaccinated 56 days later with 2 x 10^8 plaque forming units (pfu) MVA CS IM, undiluted and administered in 340µL (group 1B). Another twelve volunteers were vaccinated IM with 5 x 10^{10} vp ChAd63 CS undiluted and administered in 350µL(group 2A & 2B) and eight of these were subsequently vaccinated 56 days with 2 x 10^8 pfu MVA CS undiluted and administered in 340µL. Volunteers attended clinical follow-up at days 1, 14, 28, 56 and 90 following ChAd63 CS immunization in groups 1A and 2A and at days 1, 14, 28, 56, 57, 63, 84 and 140 following ChAd63-CS immunization in groups 1B and 2B. Safety assessments, including blood sampling for safety and immunology analysis at these visits were conducted.

Clinical Trial Authorisation was granted by the Irish Medicines Board (Case number 2107330) Ethical approval was granted by the Ethics committee of Beaumont Hospital. Vaccine use was authorized by the Environmental Protection Agency (EPA) of Ireland (Reference number G0451-01). All participants gave written informed consent prior to any study procedure being undertaken. The study was conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. The Local Safety Committee provided safety oversight and GCP compliance was independently monitored by an external organization (Appledown Clinical Research Ltd, Great Missenden, UK).

Safety

Vaccine storage was uneventful with no deviations in temperature monitoring. Vaccination storage and accountability logs were maintained and have been inspected by the EPA, Irish

Medicines Board and Appledown monitors. All vaccination events took place at the designated site and all procedures were adhered to (Standard Operating Procedures previously submitted). All GMO material and potentially contaminated material was autoclaved prior to disposal. Records of all deactivation events were kept. Leftover vaccine was returned to the University of Oxford on the 28th of January 2013.

The first volunteer to receive each vaccine at each dose was vaccinated alone and observed for 12 hours. They were then reviewed again 24 hours post vaccination. Only once 72 hours had elapsed from time of vaccination, and in the absence of safety concerns, volunteers 2 and 3 were vaccinated with that vaccine and dose. All other volunteers were observed for 30 minutes after each immunization. Prior to dose escalation of the ChAd63 CS vaccine, a report on day 14 post vaccination safety on all volunteers who received the lower dose was reviewed and approved by the independent data safety monitoring board. Volunteers were given a digital thermometer, injection site reaction measurement tool and symptom diary card to record their daily temperature, injection site reactions and solicited systemic AEs for 14 days following vaccination with ChAd63 CS and 7 days following vaccination with MVA CS. Local and systemic reactogenicity was evaluated at subsequent clinic visits and graded for severity, outcome and association to vaccination as per the criteria outlined in Tables S1-4. Blood was sampled at all visits post vaccination except days 1 and 57, and the full blood count with differential, platelet count and serum biochemistry (including electrolytes, urea, creatinine, bilirubin, alanine aminotransferase, alkaline phosphatase and albumin) measured.

Peripheral Blood Mononuclear Cell (PBMC) and Serum Preparation

Blood samples were collected into lithium heparin-treated vacutainer blood collection systems (Becton Dickinson, UK). PBMC were isolated and used within 6 hours in fresh assays. Excess cells were frozen in foetal calf serum (FCS) containing 10% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen. For serum preparation, untreated blood samples were stored at 4°C and then the clotted blood was centrifuged for 5 min (1000 xg). Serum was stored at -80°C.

Peptides for T cell Assays

Peptides (NEO Peptide, Cambridge, MA, USA) , 15 amino acids (aa) in length and overlapping by 10 aa spanning the entire CSP insert, were reconstituted in 100% DMSO at 50-200 mg/mL and combined into various pools for ELISPOT and flow cytometry assays. The composition of peptide pools containing to 2 to 15 peptides are listed in Tables 1 peptides.

***Ex-vivo* interferon-γ (IFN-γ) ELISPOT**

The kinetics and magnitude of the T cell response to CSP were assessed over time by *ex-vivo* IFN-γ ELISPOT following an 18-20 hour re-stimulation of PBMC with overlapping peptides spanning the entire CSP insert present in the viral vectored vaccines (Table 1 peptides). Fresh PBMC were used in all ELISPOT assays using a previously described protocol [1] except that CSP peptide pools (final concentration each peptide 5μg/mL) were added to test wells, culture medium was added to negative un-stimulated wells, and Staphylococcal enterotoxin B (SEB) (final concentration 20μg/mL) plus phytohemagglutinin (PHA) (final concentration 0.04μg/mL), PPD (20μg/mL) and FEC (pool of peptides from influenza, Epstein Barr virus and cytomegalovirus, final concentration 25μg/mL) was added to positive control wells. Each well contained 200,000 PBMC. Spots were counted using an ELISPOT counter (Autoimmun

Diagnostika (AID), Germany). Results are expressed as IFN-γ spot-forming units (SFU) per million PBMC. Background responses in un-stimulated control wells were almost always less than 20 spots, and were subtracted from those measured in peptide-stimulated wells. Responses are shown as the summed response to all the CSP peptide pools (unless otherwise stated).

Multiparameter Flow Cytometry

Cytokine secretion by PBMC was assayed by intracellular cytokine staining (ICS) followed by flow cytometry.

Total IgG ELISA

Anti-CSP antibodies were measured at Walter Reed Army Institute of Research, by enzyme-linked immunosorbent assay (ELISA) against the CSP repeat region using a hexameric synthetic peptide $(NANP)_6$ (CSPrp ($(NANP)_6$ Peptide [100µg/mL] Eurogentec Cat: EP070034 Lot: 14) and immunofluorescent antibody assay (IFA) using air-dried sporozoites. Details of methodology have previously been published.[2]

Statistical Analysis

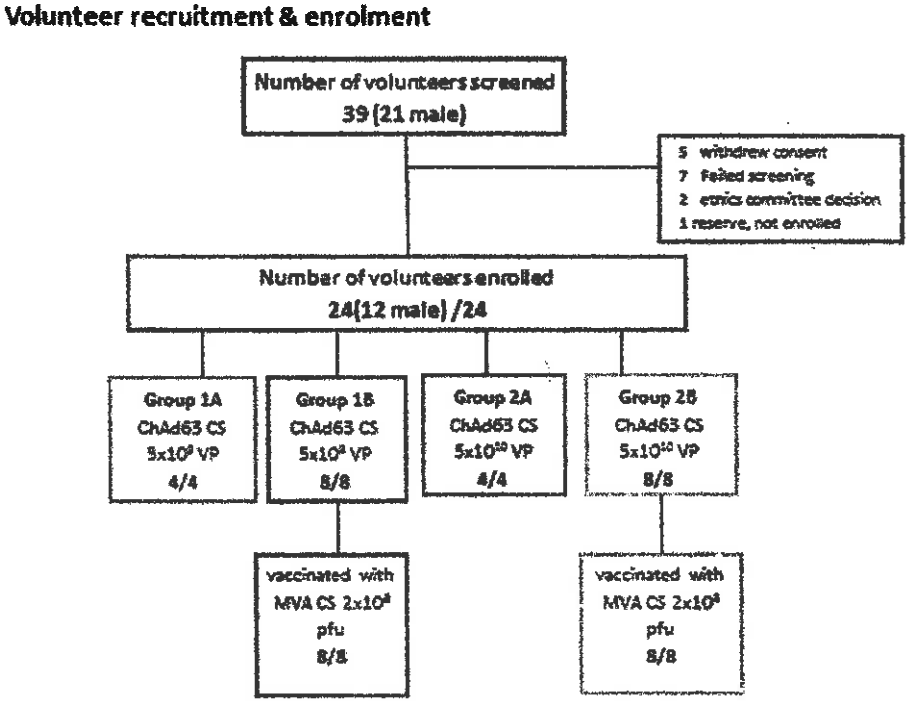
Data were analysed using GraphPad Prism version 5.04 for Windows (GraphPad Software Inc., California, USA). Geometric mean or median responses for each group are described. Significance testing of differences between two groups used the two-tailed Mann-Whitney U test or Wilcoxon signed rank test as appropriate. Correlations were analysed using Spearman's rank correlation co-efficient (r_s) for non-parametric data. A value of $P < 0.05$ was considered significant.

RESULTS

Study Recruitment

Recruitment took place between December 2011 and July 2012. Twenty four healthy malaria-naïve adult volunteers (12 female and 12 male) were enrolled, immunized and followed up (Figure 1). The mean age of volunteers was 30 years (range 21 – 46). Vaccinations began in January 2012 and all follow-up visits were completed by November 2012. All volunteers attended all visits as scheduled and completed the study.

Figure1. Volunteer recruitment & enrolment.



Safety and Reactogenicity

No unexpected or serious AEs occurred and no volunteers were withdrawn due to AEs.

ChAd63 CS demonstrated a good safety profile with the majority of AEs being mild in severity (91%) and 80% of all AEs resolved within 48 hours. (Figure 2 Duration of AEs) Overall, 14 out of 24 (58%) experienced one or more local AEs related to ChAd63 CS; all were mild. 20 out of 24 (83%) experienced one or more systemic AE related to ChAd63 CS. Significant difference was observed comparing ChAd63 CS at the lower dose with higher dose in terms incidence of local pain ($p = 0.04$ Mann-Whitney test) but duration of pain was not ($P = 0.15$ Mann-Whitney test). ChAd63 CS at the higher dose was more likely to be associated with systemic AEs (P=0.03). Looking at systemic AEs categories separately, only arthralgia showed a significant difference between low and high dose, (p=0.036 Mann-Whitney). All systemic AEs related to the low dose ChAd63 CS were mild, however, one volunteer who received high dose ChAd63 CS reported severe fatigue. MVA CS was administered 8 weeks after the ChAd63 CS and was more reactogenic, with 14 out of 16 (87%) experiencing local AEs, mainly pain, erythema and warmth. All but one of these AEs was mild or moderate. One episode of severe injection site pain was recorded, which resolved within 48 hours. 15 out of the 16, (93%) experienced a combination of systemic AEs, including feverishness, myalgia, fatigue, malaise and headache in the 24 hours following vaccination. All were mild other than three exceptions of severe AEs; one each of fever, myalgia and headache, which were all experienced by one volunteer simultaneously and resolved within 48 hours of vaccination. This is similar to the flu-like symptoms that have been reported in the past with MVA vaccines expressing other antigens.[1,3-5]

One moderate laboratory AE of neutropenia was observed following ChAd63 CS at the lower dose of $5x10^9$ vp. This has been previously described following vaccination.[6] Screening results were within range, but at the lower end at 1.56×10^9/L (normal range 1.56 – $6.52x10^9$/L). Two weeks vaccination with ChAd63 CS their neutrophil count was $1.58 x10^9$/L.

At next review this dropped to $1.09x10^9$/L, total WCC $3.57x10^9$/L. At last review neutrophil count was 1.15 x 10^9/L (Nov 2012). Investigations for other causes of neutropenia were normal. The volunteer was clinically well at end of study and has been referred to a haematology service for review. It is our opinion that this volunteer had a low neutrophil count at screening and the AE recorded is unlikely to be related to the vaccine, however it was decided to declare this AE as possibly related so that it could be fully reported.

Figure 2. The mean duration and range of AEs following each vaccine is shown. Figure X(a) local AEs and Fig. X(b) systemic AEs following ChAd63 CS $5x10^9$ vp; figure X(c) local and figure X(d) systemic AEs following ChAd63 CS $5x10^{10}$ vp; figure X(e) local and figure X(f) systemic AEs following MVA CS.

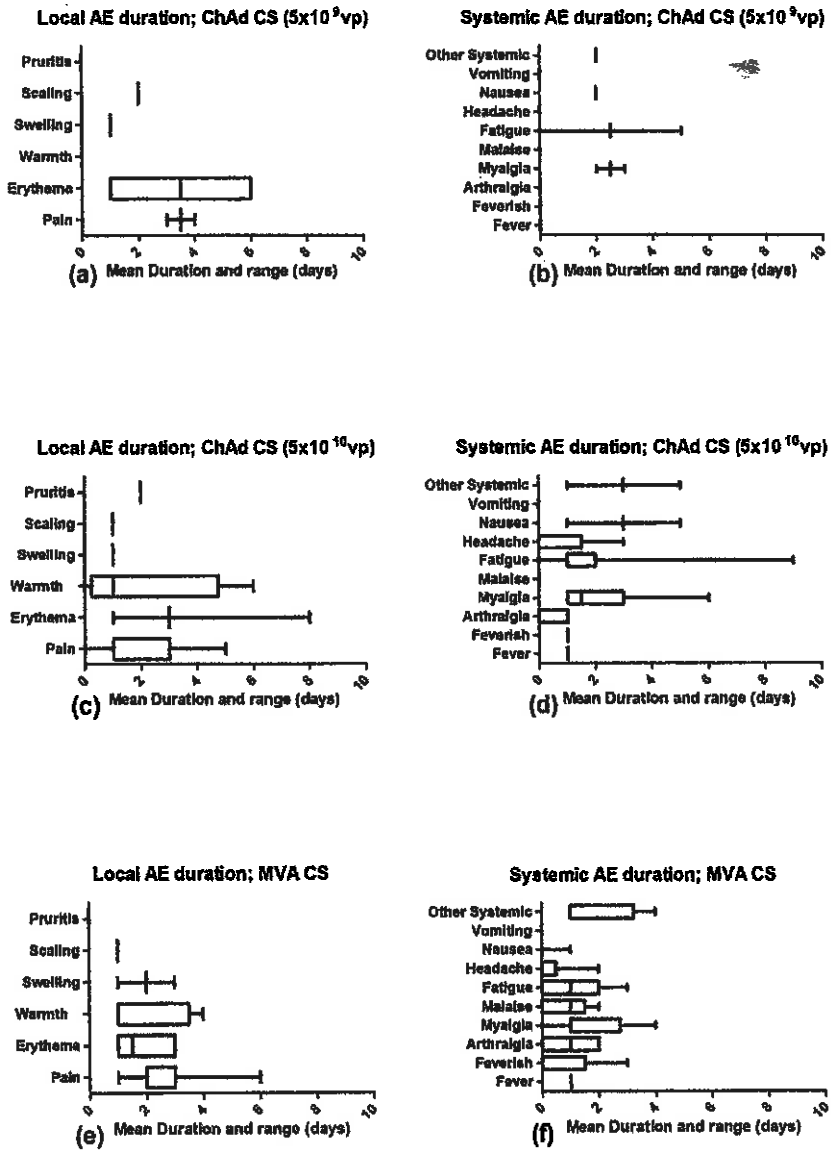
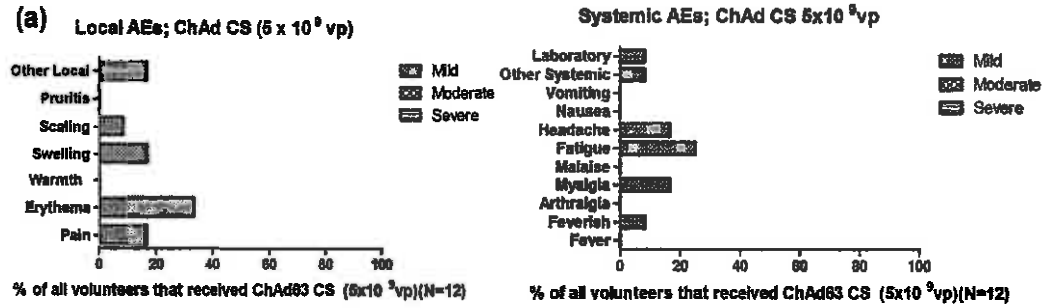
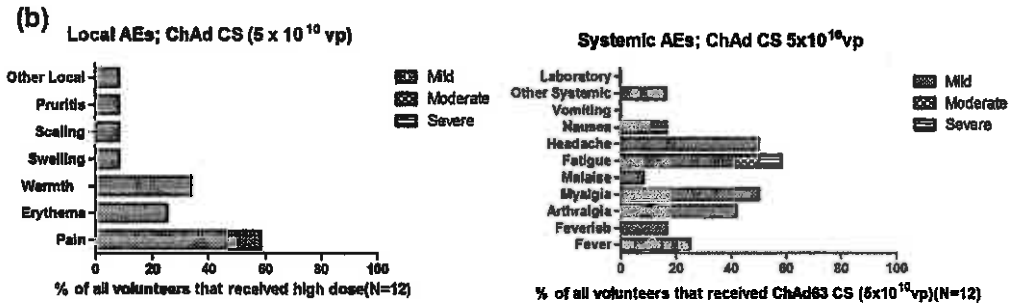
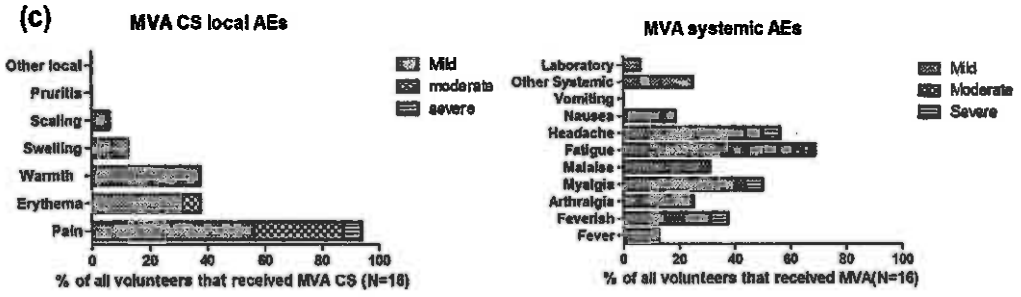


FIGURE 3. Local and systemic adverse events (AEs) following vaccination are shown as percentage of volunteers. (a) shows local and systemic AEs following administration of ChAd63 CS 5 x 10^5 vp; (b) shows local and systemic AEs following administration of ChAd63 CS 5 x10^{10} vp; (c) shows local and systemic AEs following administration of MVA CS.





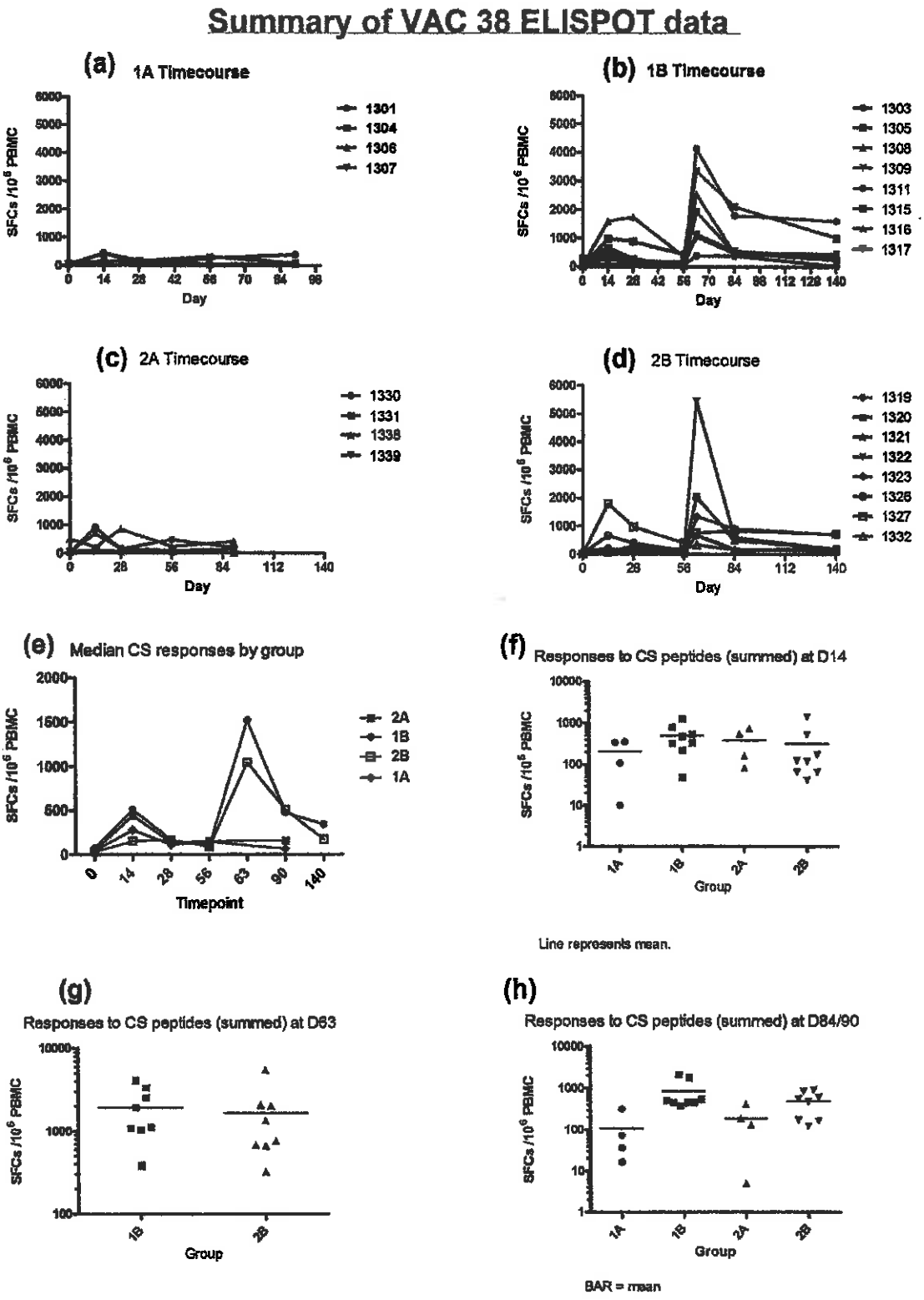


ChAd63-MVA CS T cell immunogenicity assessed by *ex-vivo* IFN-γ ELISPOT

Antigen-specific T cell responses in all volunteers as measured by *ex-vivo* IFN-γ ELISPOT are shown in Figure 4 and median responses to the total vaccine insert are shown for each

group in Figure 4(e). When comparing the responses to two different doses of ChAd63 CS , no significant difference was seen between group 1 (lower dose) and group 2 (higher dose) at the peak of the response on day 14 (median 423 mean 500 [range 12.5 – 1590] vs 178 mean 426 [range 52.5 – 1795] SFU/million PBMCs in groups 1 versus 2 respectively, n = 12 v 12, P = 0.54 by Mann-Whitney test). Thereafter T cell responses gradually contracted to day 56 (Figure 4). Administration of MVA CS at day 56 significantly boosted responses in all volunteers as measured 7 days later on day 63 (Figure 4). No statistical difference was seen comparing those who had first received the low dose ChAd63 CS, group 1B, with those who had received the higher dose ChAd63 CS, group 2B, (median 1523, mean 1947 [range 380 – 4125] vs 1048 mean 1659 [range 320 – 5450] SFU/million PBMCs in groups 1B and 2B respectively, n=8 v 8, P = 0.5 by Mann-Whitney test). Responses contracted but remained above baseline at the last time-point, day 140, (median 1B 346.25 mean 537 [range 17.5 – 1607], median 2B 176 mean 299 [range 102 – 707] SFU/million PBMCs), Figure 4. There was no significant difference between the groups at this stage (p = 0.16 by Mann-Whitney test).

Figure 4. Summary of ELIspot responses of volunteers in ea ch group. Summed SFU / million



N.B. No significant difference between groups and doses at any timepoint.

CSP T cell multi-functionality

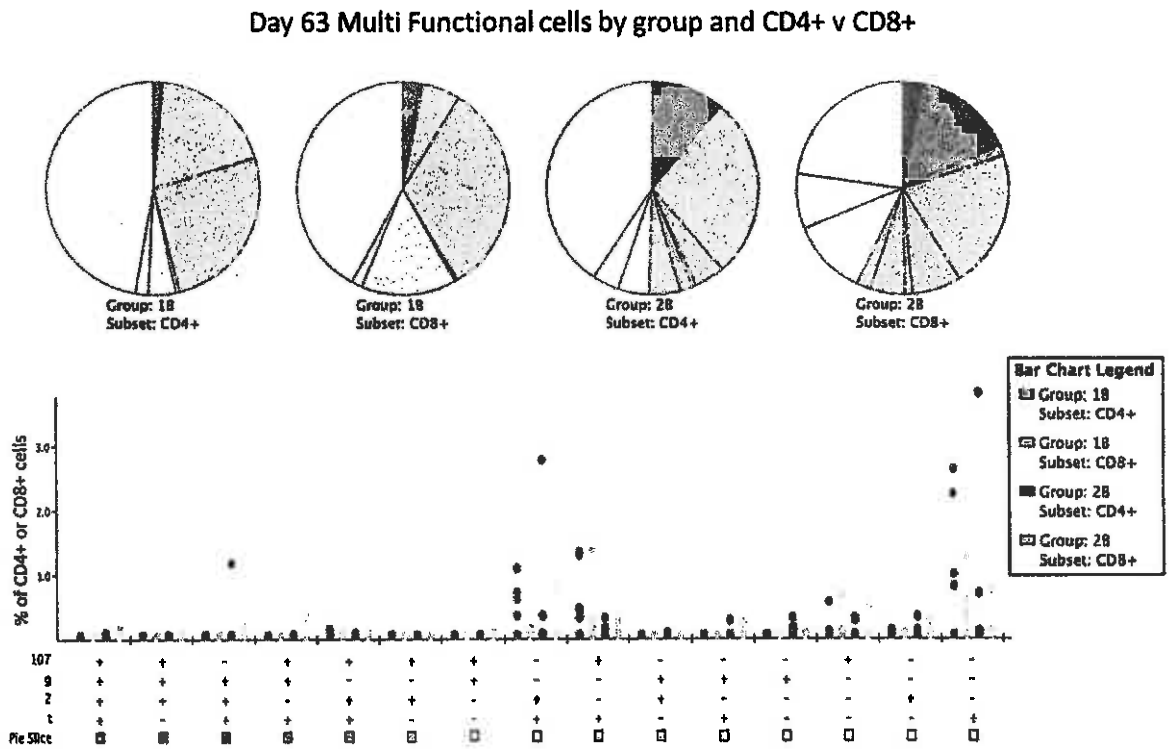
Antigen-specific $CD3^+$ T cell functionality was also assayed by ICS at the days 14, 63 and 84/90 time-points (Figure MF cells graphics). Following peptide re-stimulation, detectable CSP-specific $CD3^+$ T cells consisted of a mixed $CD4^+$ and $CD8^+$ phenotype. It should be noted that the ELISspot and ICS assays vary in methodology (including the use of multiple versus a single peptide pool respectively, as well as differences in peptide concentration, use of co-stimulatory antibodies and use of fresh versus frozen PBMC).

Day 63 Multi-functional cells. Percent of parent population (CD4+ or CD8+) producing 1 or more cytokine by group.					
		4 cytokines	3 cytokines	2 cytokines	1 cytokine
1B	CD4	0.0000	0.0277	1.0346	3.5630
	CD8	0.0019	0.0186	0.2990	0.9205
2B	CD4	0.0055	0.1566	0.6155	1.960
	CD8	0.0153	0.0704	0.2080	1.0059

Across all three time-points analysed CD107a (marker of degranulation) expression was upregulated by both $CD4^+$ and $CD8^+$ T cells. $CD4^+$ cells produced higher levels of TNFα than $CD8^+$ cells at all time-points, but this was not statistically significant ($P = 0.58$, $P = 0.31$ and $P = 0.48$ for days 14, 63 and 84 respectively, Mann-Whitney test). $CD4^+$ cells also produced greater levels of IL-2 compared to $CD8^+$ cells at all time-points, however this did not reach significance ($P = 0.77$, $P = 0.59$ and $P = 0.32$ for days 14, 63 and 84 respectively, Mann-Whitney test). Negligible levels of IFN-γ were produced by either CD4+ or CD8+ cells at days

14 and 63. Levels comparable to IL-2 and TNFα were observed at day 84. Distinct populations of $CD4^+$ and $CD8^+$ T cells expressing 1+, 2+, 3+ or 4+ functional markers / cytokines were evident following a Boolean gate analysis (Figure 5 pie chart of MFN and table of percentage cells producing 1-4 markers).

Figure 5.

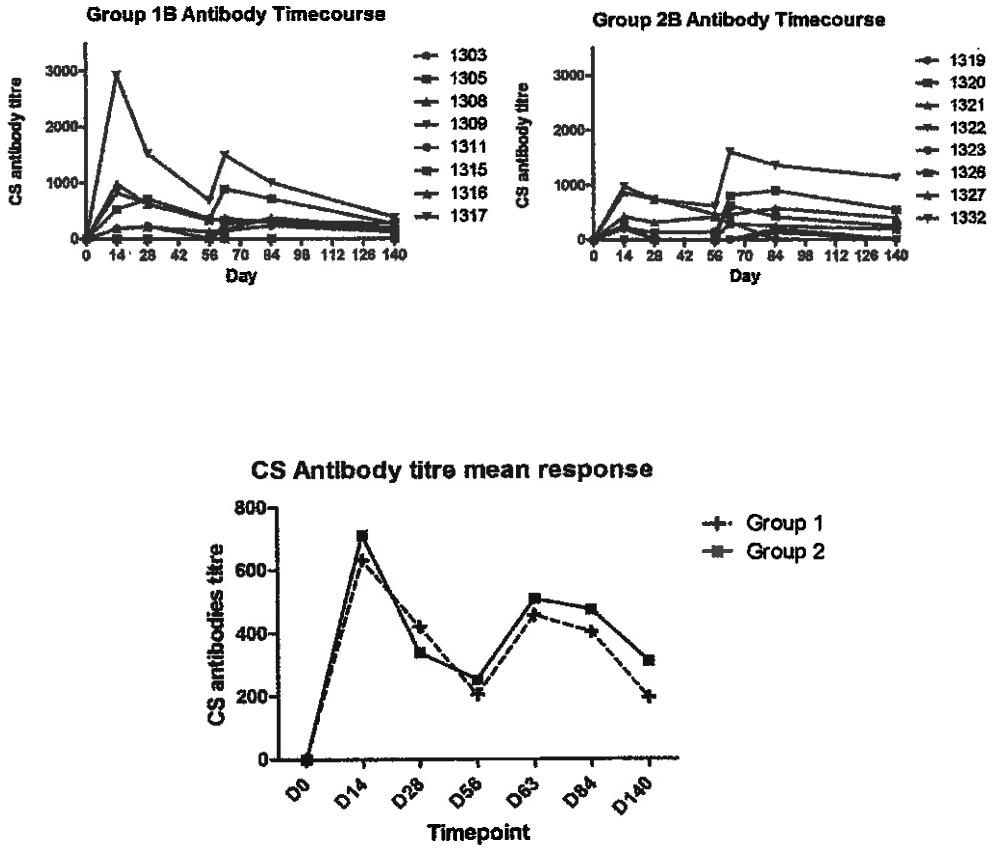


ChAd63-MVA CS antibody immunogenicity assessed by ELISA

The kinetics and magnitude of the serum IgG antibody response against the CSP were assessed over time by ELISA. CSP-specific IgG was induced in all volunteers, with individual responses and geometric mean shown in Figure 6 each group. All volunteers had IgG titres below the limit of detection at day zero. Mean responses peaked at day 14 (3μg/ml) for both doses of ChAd63 CS and did not differ significantly between groups (P = 0.95 Mann-Whitney test). Boosting with MVA CS resulted in a significant increase in antibody concentration in group 1B, compared to the unboosted

group 1A, as measured at day 84/90 (P = 0.037 Mann-Whitney test). However this was not seen when comparing groups 2A and 2B at the same time-points (P = 0.49 Mann-Whitney test). Mean antibody response was higher in group 2B compared to group 1B at day 140, but was not significant (P = 0.87 Mann-Whitney test).

Figure 6.



Post-release evaluation of the risks to human health and the environment

Here we have shown in a phase I, first administration into humans, that ChAd63-CS and MVA–CS have an acceptable safety profile, and are immunogenic, inducing antigen-specific multifunctional CD4+ and CD8+ T lymphocytes, and significant levels of antibody.

No serious adverse events occurred during the course of the trial. The majority of AEs observed were mild in intensity and resolved rapidly. Over 300 healthy volunteers have

now received ChAd63 encoding the malaria antigens; ME-TRAP, MSP1 and AMA1. The safety profile seen with ChAd63 CS was very similar to that of other ChAd63 vectored vaccines. [1,5,7-9] The safety profile observed following administration of MVA CS was also similar to that reported following MVA expressing different antigens.[5,7] [1,9] The majority of volunteers receiving this vector experience a constellation of symptoms comprising of feverishness, fatigue, headache and myalgia. These are of mild severity in the majority of cases and all resolve within 48 hours. The safety data collected in this study adds to the already significant body of data supporting the excellent safety profile of this vaccine delivery platform. These vaccines and other similar ones utilizing similar viral vectors appear to be safe for the wider environment. As per the initial application submitted to the EPA, 29th July 2011, these vaccines are replication deficient, lacking the E1 locus. They have not been shown to pose a risk to the wider environment and in this study an acceptable human safety profile has been shown.

A statement on the results of the clinical trial in relation to any product, or type of product, in respect of which consent to placing on the market may be sought.

Following this study in Ireland the vaccine has been studied in a phase II trial in the UK. A further 15 human volunteers received the vaccines, ChAd63 CS and MVA CS. The safety profile was very similar to that seen in this study, there were no severe AEs or SAEs. The vaccine showed some clinical efficacy against controlled human malaria challenge and further studies are on-going. There are no plans at this time to seek consent for placing these vaccines on the market.

Signed:

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References

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Table 1, peptide pools used for Ellspot and Flow Cytometry.

PEPTIDE	SEQUENCE	Pool	aa sequence
CSN1	MRKLAILSVSSFLFV		1-40
CSN2	ILSVSSFLFVEALFQ	1	
CSN3	SFLFVEALFQEYQCY	6-peptides	
CSN4	EALFQEYQCYGSSSN		
CSN5	EYQCYGSSSNTRVLN		
CSN6	GSSSNTRVLNELNYD		
CSN7	TRVLNELNYDNAGTN		30-70
CSN8	ELNYDNAGTNLYNEL	2	
CSN9	NAGTNLYNELEMNYY	6-peptides	
CSN10	LYNELEMNYYGKQEN		

CSN11	EMNYYGKQENWYSLK		
CSN12	GKQENWYSLKKNSRS		
CSN13	WYSLKKNSRSLGEND	3	60-105
CSN14	KNSRSLGENDDGNNE		
CSN15	LGENDDGNNEDNEKL	7 - peptides	
CSN16	DGNNEDNEKLRKPKH		
CSN17	DNEKLRKPKHKKLKQ		
CSN18	RKPKHKKLKQPADGN		
CSN19	KKLKQPADGNPDPNA		
CSN20	PADGNPDPNANPNVD	4	95-180
CSN21	PDPNANPNVDPNANP		
CSN22	NPNVDPNANPNVDPN	15 - peptides	
CSN23	PNANPNVDPNANPNV		
CSN24	NVDPNANPNVDPNAN		
CSN25	ANPNVDPNANPNANP		
CSN26	DPNANPNANPNANPN		
CSN27	PNANPNANPNANPNA		
CSN28	NANPNANPNANPNAN		
CSN29	ANPNANPNANPNANP		
CSN30	NPNANPNANPNANPN		
CSN31	PNANPNANPNANPNA		
CSN32	NANPNANPNANPNKN		
CSN33	ANPNANPNKNNQGNG		
CSN34	NPNKNNQGNGQGHNM		
CSN35	NQGNGQGHNMPNDPN	5	170-210
CSN36	QGHNMPNDPNRNVDE		
CSN37	PNDPNRNVDENANAN	6 - peptides	
CSN38	RNVDENANANSAVKN		
CSN39	NANANSAVKNNNNEE		
CSN40	SAVKNNNNEEPSDKH		
CSN41	NNNEEPSDKHIKEYL	6	200-225
CSN42	PSDKHIKEYLNKIQN	3 - peptides	
CSN43	IKEYLNKIQNSLSTE		
CSN44	NKIQNSLSTEWSPCS	7	215-250
CSN45	SLSTEWSPCSVTCGN		
CSN46	WSPCSVTCGNGIQVR	5 - peptides	
CSN47	VTCGNGIQVRIKPGS		
CSN48	GIQVRIKPGSANKPK		
CSN49	IKPGSANKPKDELDY	8	240-260
CSN50	ANKPKDELDYANDIE	2 - peptides	
CSN51	DELDYANDIEKKICK	9	250-285
CSN52	ANDIEKKICKMEKCS		
CSN53	KKICKMEKCSSVFNV	5 - peptides	
CSN54	MEKCSSVFNVVNSSI		
CSN55	SVFNVVNSSIG		

Table S1: *Guidelines for assessing the relationship of vaccine administration to an AE*

0	**No Relationship**	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
1	**Unlikely**	Unlikely temporal relationship to study product ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to study product.
2	**Possible**	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
3	**Probable**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
4	**Definite**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

Table S2: *Severity grading criteria for adverse events*

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
Erythema at injection site*	1	>3 - ≤50 mm
	2	>50 - ≤100 mm
	3	>100 mm
Swelling at injection site	1	>3 - ≤50 mm
	2	>50 - ≤100 mm
	3	>100 mm
Fever (oral)	1	37.6°C - 38.0°C
	2	>38.0°C – 39.0°C
	3	>39.0°C
Headache	1	Headache that is easily tolerated
	2	Headache that interferes with daily activity
	3	Headache that prevents daily activity
Nausea	1	Nausea that is easily tolerated

	2	Nausea that interferes with daily activity
	3	Nausea that prevents daily activity
Malaise	1	Malaise that is easily tolerated
	2	Malaise that interferes with daily activity
	3	Malaise that prevents daily activity
Myalgia	1	Myalgia that is easily tolerated
	2	Myalgia that interferes with daily activity
	3	Myalgia that prevents daily activity
Arthralgia	1	Joint pain that is easily tolerated
	2	Joint pain that interferes with daily activity
	3	Joint pain that prevents daily activity
Urticaria	1	Requiring no medications
	2	Requiring oral or topical treatment or IV medication or steroids for <24 hours
	3	Requiring IV medication or steroids for >24 hours

**erythema ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event*

Table S3: *Severity grading criteria for laboratory abnormalities*

Laboratory Test	Grade 1	Grade 2	Grade 3
Hgb (female) – decrease from testing laboratory LLN in gm/dl	>1.0 - <1.5	≥1.5 & <2.0	≥2.0
Hgb (male) – decrease from testing laboratory LLN in gm/dl	≥1.5 & <2.0	≥2.0 & <2.5	≥2.5
Absolute neutrophil count (ANC, cells/mm^3)	1000-1499	500-999	<500
Leukopenia (WBC, cells/mm^3)	<3500 - ≥2500	<2500 - ≥1500	<1500
Platelets (cells/mm^3)	125,000 – 135,000	100,000 – 124,000	20,000-99,000
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN
ALT	1.25 – 2.5 x ULN	>2.6 – 5.0 x ULN	>5.0 x ULN
Creatinine	1.1 – 1.5 x ULN	>1.6 – 3.0 x ULN	>3.0 x ULN
Urine protein	2+ or 0.5-1 gm loss/day	3+ or 1-2 gm loss/day	4+ or >2 gm loss/day
Hematuria	2+ confirmed by 5-10 rbc/hpf	3+ confirmed by >10 rbc/hpf	gross, with or without clots, OR red blood cell casts

Table S4: *Functional scale for assessing the severity of AEs*

Scale	Description	Definition
1	Mild	Awareness of a symptom but the symptom is easily tolerated
2	Moderate	Discomfort enough to cause interference with usual activity
3	Severe	Incapacitating; unable to perform usual activities; requires absenteeism or bed rest