

Novel Viral Vector Vaccines for Tropical Infections

Thesis submitted for the degree of Doctor of Philosophy
Trinity Term 2017

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ABSTRACT

Plasmodium falciparum (*P.falciparum*) malaria accounts for over 200 million cases annually and over 400 000 deaths. In 2014 Ebolavirus disease (EVD) caused a devastating outbreak in West Africa, resulting in over 28 000 cases and 11 000 deaths. At present, no licensed vaccine exists for either tropical infection. Viral vectors are showing great promise as a novel vaccine platform technology, having been shown to induce both cellular and humoral immune responses. Malaria vaccine efficacy can be assessed using controlled human malaria infection (CHMI) studies, in which healthy volunteers are infected with *P. falciparum* and then followed up for development of clinical disease. No such human challenge model exists for EVD.

This thesis describes 3 clinical trials. Two studies evaluate the safety, efficacy and immunogenicity of a combination vaccination regimen containing the leading pre-erythrocytic *P. falciparum* malaria vaccine RTS,S/AS01B and the liver stage, viral vector heterologous prime-boost regimen of ChAd63 ME-TRAP and MVA ME-TRAP. Vaccine efficacy is evaluated using the CHMI model. One trial evaluates a regimen in which immunisation with RTS,S/AS01B and ChAd63/MVA ME-TRAP is chronologically staggered. The other evaluates concomitant administration of the vaccines, and is

also the first study to evaluate the effect of a reduced third dose of RTS,S/AS01B when given on a 0, 1, 2-month schedule. The third study presented here is a first in man study describing the safety and immunogenicity of the viral vector Ebolavirus vaccine, ChAd3-EBO Z either given alone or in a heterologous prime-boost sequence with MVA-BN® Filo, for which this study is also a first in man evaluation.

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and Philipps-Universität Marburg.

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PUBLICATIONS ARISING FROM THIS WORK

- **Rampling T**, Ewer K, Bowyer G *et al*. Safety and High Level Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP. J Infect Dis. 2016 Jun 15. pii: jiw244. PMID: 27307573
- Ewer K*, **Rampling T*,** Venkatraman N* *et al.* A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. N Engl J Med. 2016 Apr 28;374(17):1635-46. PMID: 25629663 *Equal contribution – joint first author. Follow up to preliminary report (below)
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- **Rampling T**, Ewer K, Hill AVS *et al*. Safety and Efficacy of Novel Malaria Vaccine Regimens of RTS,S/AS01B alone, or with Concomitant ChAd-MVA Vectored Vaccines Expressing ME-TRAP.

Table of Contents

ABSTRACT 2
ACKNOWLEDGEMENTS 4
PUBLICATIONS ARISING FROM THIS WORK 6
1. Chapter 1: Introduction 23
1.1. Rationale for Thesis 23
1.2. Diseases of the Tropics 23
1.3. Vaccines for Tropical Diseases 24
1.4. Malaria 24
1.4.1. Epidemiology and Global Burden of Disease 26
1.4.2. Malaria Transmission Dynamics 26
1.4.3. Life Cycle and Pathogenesis 27
1.4.4. *Plasmodium Falciparum* Malaria 30
1.4.5. Attempts at global control and potential elimination of malaria 31
1.4.6. History, and Current Status of Malaria Vaccine Development 33
1.4.7. The Pre-Erythrocytic Stage of Malaria Infection as a Vaccine Target 34
1.4.8. RTS,S/AS01B 35
1.4.9. Viral Vector Vaccines 41
1.4.10. Adenovirus Vectored Vaccines 42
1.4.11. MVA Vectored Vaccines 45
1.4.12. ME-TRAP 47
1.4.13. Concept of a Multi-Stage/Multi-component Malaria Vaccine 52
1.5. Microbial Challenge Studies of Human Subjects 54
1.5.1. Controlled Human Malaria Infection (CHMI) 55
1.5.2. CHMI Delivered by Mosquito Bite 56
1.5.3. Conduct of CHMI Trials 57
1.5.4. Ethical Considerations of CHMI Trials 59
1.5.5. Clinical Presentation Following CHMI 60
1.6. Ebola 62
1.6.1. History of Ebola Virus Discovery 62
1.6.2. Virology and Pathogenesis 62
1.6.3. The 2014 West African Ebola Outbreak 64
1.6.4. The need for an Ebola vaccine 64

1.6.5. Development of Ebola Vaccines 65
1.6.6. Previous Experience with VRC Filovirus Vaccines 65
1.6.7. ChAd3-EBO Z 67
1.6.8. Previous Experience with Chimpanzee Adenovirus Vector Serotype 3 (ChAd3) 68
1.6.9. MVA-BN® Filo 69
1.6.10. Rationale 70
1.7. Aim of Work 71
1.8. Overview of Thesis Chapters 73
2. Chapter 2: Materials and Methods 75
2.1. Investigational Medicinal Products (IMPs) 75
2.1.1. Manufacturing and Presentation of Vaccines 75
2.2. MVA-BN® Filo 77
2.3. Treatment of Trial Volunteers 79
2.3.1. Recruitment & Withdrawal of Trial Volunteers 79
2.3.2. Informed Consent 81
2.3.3. Inclusion and Exclusion Criteria 82
2.3.4. Effective contraception for female volunteers 87
2.3.5. Prevention of “over-volunteering” 87
2.3.6. Compensation of volunteers 87
2.3.7. Exclusion criteria at the time of vaccination 88
2.3.8. Exclusion criteria on Day of CHMI (VAC055 & VAC059 only) 89
2.3.9. Withdrawal of Volunteers 89
2.4. Criteria for dose continuation and dose escalation 90
2.4.1. Stepwise Vaccination Process for Concomitant Administration of RTS,S/AS01B with ChAd63 or MVA ME-TRAP 90
2.4.2. EBL01: Enrolment of Group 1 (ChAd3-EBO Z 1 x 10^{10}vp) 90
2.4.3. EBL01: Enrolment of group 2 (2.5 x 10^{10}vp) 90
2.4.4. EBL01: Enrolment of group 3 (5 x 10^{10}vp) 91
2.4.5. Initiation of vaccinations with MVA-BN® Filo in Groups 1-3 92
2.4.6. EBL01: Enrolment of Groups 4-7 92
2.5. Safety stopping and holding rules 93
2.6. Treatment of Trial Volunteers 93
2.6.1. Trial Sites 93

2.6.2. Study Procedures 94
2.6.3. Screening Visit 94
2.6.4. Blood Tests, oral fluid tests, urinalysis and electrocardiograms 95
2.6.5. Administration of Vaccines 96
2.6.6. Day of Vaccination 99
2.6.7. Clinical Reviews 100
2.7. Controlled Human Malaria Infection Procedures (VAC055 & VAC059 trials only) 100
2.7.1. Day before CHMI (C-1) 100
2.7.2. Day of CHMI (C0) 100
2.7.3. CHMI Procedure 101
2.7.4. Days 1-5 post CHMI (C1 - C5) 103
2.7.5. Days 6.5 – Day 23 (VAC055) or Day 25 (VAC059) Post CHMI (C6.5 – C23/C25) 103
2.7.6. Malaria Diagnosis 104
2.7.7. Malaria Parasite Quantitative PCR (qPCR – VAC055 & VAC059 only) 105
2.7.8. Malaria Management 106
2.7.9. Follow Up Post Diagnosis 106
2.7.10. Post CHMI visits (C+35 onwards) 107
2.7.11. Re-Challenge of Protected Vaccinees (VAC055 only) 107
2.8. Assessment of Safety 107
2.8.1. Definitions 108
2.8.2. Causality Assessment 110
2.8.3. Additional Safety Oversight 112
2.9. Immunological Assays 113
3. Safety, Efficacy and Immunogenicity of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP (VAC055) 115
3.1. Authorship Statement 115
3.2. Introduction 116
3.3. Objectives 116
3.3.1. Primary Objective: 116
3.3.2. Secondary Objectives: 117
3.3.3. Tertiary Objectives: 117

3.4. Participants 117
3.5. Ethical and Regulatory Approval 118
3.6. Study Design 119
3.7. Results 121
3.7.1. Participants 121
3.7.2. Protective efficacy against CHMI 122
3.7.1. Parasite Density Endpoints: 127
3.7.2. Safety 131
Figure 17: Solicited reactogenicity in the 7 day period following vaccination with ChAd63 ME-TRAP 137
3.7.3. Immunogenicity 139
3.8. Discussion 150
4. Safety and Efficacy of Novel Malaria Vaccine Regimens of RTS,S/AS01B alone, or with Concomitantly Administered ChAd-MVA Vectored Vaccines Expressing ME-TRAP (VAC059) 157
4.1. Authorship Statement 157
4.2. Introduction 158
4.3. Objectives 159
4.3.1. Primary Objective: 159
4.3.2. Secondary Objectives: 159
4.3.3. Tertiary Objectives: 160
4.4. Participants 160
4.5. Ethical and Regulatory Approval 161
4.6. Study Design 161
4.7. Results 163
4.7.1. Participants 163
4.7.2. Protective efficacy against CHMI 165
4.7.3. Safety 172
4.7.4. Immunogenicity 180
4.8. Discussion 199
5. A Phase Ia, Dose-Escalating, Safety and Immunogenicity trial of the monovalent Zaire Ebola viral vector candidate vaccine ChAd3-EBO Z and the heterologous prime-boost candidate vaccine regimen ChAd3-EBO Z and MVA-BN® Filo in healthy UK adults (EBL01) 207
5.1. Authorship Statement 207

5.2. Introduction 208
5.3. Objectives 209
5.3.1. Primary Objective: 209
5.3.2. Secondary Objectives: 209
5.4. Participants 209
5.5. Ethical and Regulatory Approval 210
5.6. Study Design 210
5.6.1. Study Groups 211
5.7. Results 214
5.7.1. Participants 214
5.7.2. Safety 217
5.7.3. Immunogenicity 228
5.8. Discussion 248
6. Conclusions and Closing Remarks 254
6.1. Viral vectored vaccines for tropical infections 254
6.2. Anti-vector immunity 255
6.3. Implications for malaria vaccines 256
6.4. Implications for Ebola vaccines 258
7. APPENDIX 260
7.1. Schedule of procedures (ie. Trial visits blood tests etc.) 260
7.1.1. Schedule of Procedures for VAC055 (Chapter 3) 260
7.1.2. Schedule of Procedures for VAC059 (Chapter 4) 266
7.1.3. Schedule of Procedures for EBL01 (Chapter 5) 271
7.2. Immunology & PCR methods 277
7.2.1. Malaria Immunogenicity Assays (VAC055 and VAC059) 277
7.2.2. Ebola Immunogenicity Assays (EBL01) 288
7.3. Safety Appendix 294
7.3.1. VAC055: Solicited Adverse Events 294
7.3.2. VAC055 Unsolicited Adverse Events 296
7.3.3. VAC055 Laboratory Adverse Events 304
7.3.4. VAC059 Solicited Adverse Events 307
7.3.5. VAC059 Unsolicited Adverse Events 310
7.3.6. EBL01 Unsolicited Adverse Events 319
8. References 327

List of Figures

Figure 1: Malaria parasite life cycle..... 28
Figure 2: MAL034: Kaplan-Meier log rank comparisons 48
Figure 3: VAC045 clinical trial: Kaplan-Meier comparisons..... 49
Figure 4: MAAL034 immunogenicity/efficacy associations..... 51
Figure 5: EBL01: Pathway for dose escalation and vaccination..... 92
Figure 6: VAC055 Flow of study design and volunteer recruitment. 122
Figure 7: VAC055 Kaplan-Meier survival analyses of primary endpoint 124
Figure 8: VAC055 Kaplan-Meier survival analyses of secondary endpoint. 125
Figure 9: VAC055 Kaplan-Meier survival analyses of secondary endpoint 126
Figure 10: VAC055 Comparison of mean parasite densities 129
Figure 11: VAC055 Group mean log-transformed PCR data..... 129
Figure 12: VAC055 AUC analysis of parasite densities 130
Figure 13: VAC055 Kaplan-Meier survival analyses of tertiary endpoint..... 131
Figure 14: VAC055 Solicited reactogenicity following 1st dose of RTS,S/AS01B..... 134
Figure 15: VAC055 Solicited reactogenicity following 2nd dose of RTS,S/AS01B..... 135
Figure 16: VAC055 Solicited reactogenicity following 3rd dose of RTS,S/AS01B 135
Figure 17: VAC055 Solicited reactogenicity following ChAd63 ME-TRAP 137
Figure 18: VAC055 Solicited reactogenicity following MVA ME-TRAP..... 138
Figure 19: VAC055 Anti-CS IgG antibody responses 140
Figure 20: VAC055 Comparison of Anti-CS IgG antibody responses..... 141
Figure 21: VAC055 Correlation between anti-CS IgG and parasite density 141
Figure 22: VAC055 anti-CS IgG avidity..... 142
Figure 23: VAC055 Median anti-TRAP IgG responses 142
Figure 24: VAC055 Median T-cell responses to ME-TRAP..... 144
Figure 25: VAC055 Median T-cell responses to CSP..... 144
Figure 26: VAC055 ICS analysis 1..... 145
Figure 27: VAC055 ICS analysis 2..... 146
Figure 28: VAC055 ICS analysis 3..... 146
Figure 29: VAC055 ICS analysis 4..... 148
Figure 30: VAC055 ICS analysis 5..... 148
Figure 31: VAC055 ICS analysis 6..... 149

Figure 32: VAC055 CSP ELISpot responses by peptide pool 149
Figure 33: VAC059 Flow of study design and volunteer recruitment. 164
Figure 34: VAC059 Kaplan-Meier survival analyses of primary endpoint 168
Figure 35: VAC059 Kaplan-Meier survival analyses of primary endpoint 168
Figure 36: VAC059 Kaplan-Meier survival analyses of secondary endpoint 169
Figure 37: VAC059 Kaplan-Meier survival analyses of secondary endpoint 170
Figure 38: VAC059 Solicited reactogenicity following 1st vaccination 174
Figure 39: VAC059 Solicited reactogenicity following 2nd vaccination 175
Figure 40: VAC059 Solicited reactogenicity following 3rd vaccination..... 175
Figure 41: VAC059 Median Anti-NANP IgG time courses..... 183
Figure 42: VAC059 Anti-NANP IgG responses key timepoints..... 184
Figure 43: VAC059 C-terminal IgG responses after each RTS,S vaccination 184
Figure 44: VAC059 IgG responses against full-length CSP..... 185
Figure 45: VAC059 Avidity of total IgG against full-length CSP..... 185
Figure 46: VAC059 Avidity of total IgG, IgG1 and IgG3 against NANP 186
Figure 47: VAC059 TRAP-specific IgG responses..... 186
Figure 48: VAC059 (vs. VAC055) Median TRAP IgG time courses..... 187
Figure 49: VAC059 Total IgG against MVA..... 189
Figure 50: VAC059 Fold-change in anti-MVA antibody 189
Figure 51: VAC059 Anti-MVA IgG responses by protection status. 190
Figure 52: VAC059 Relationship between anti-MVA antibodies & T-cells 190
Figure 53: VAC059 (vs. VAC055) Anti-MVA antibodies & T-cells..... 191
Figure 54: VAC059 Median ELISPOT CSP 193
Figure 55: VAC059 Median ELISPOT response to ME-TRAP 194
Figure 56: VAC059 ME-TRAP-specific T cell responses 194
Figure 57: VAC059 ICS responses to CSP peptides..... 195
Figure 58: VAC059 ICS responses to TRAP peptides 195
Figure 59: VAC059 ICS CD8+ T cell responses to TRAP..... 196
Figure 60: VAC059 CS-specific IgG responses by protection status..... 198
Figure 61: VAC059 CSP-specific IgG & TRAP specific T-cells by protection status.... 198
Figure 62: VAC059 CSP-specific IgG & TRAP specific T-cells by protection status.... 199
Figure 63: EBL01 Pathway for dose escalation and vaccination..... 213
Figure 64: EBL01 flow chart of study design and volunteer recruitment 215
Figure 65: EBL01 Solicited reactogenicity following ChAd3-EBO Z vaccination. 223

Figure 66: EBL01 Solicited reactogenicity following MVA-BN® Filo vaccination 225
Figure 67: EBL01 Anti-GP titres 231
Figure 68: EBL01 Individual anti-GP responses post ChAd 3 EBO Z 232
Figure 69: EBL01 GMTs of anti-GP responses by dose group 232
Figure 70: EBL01 post-prime/Post boost anti-GP responses 233
Figure 71: EBL01 NIH EC90 ELISA titres 233
Figure 72: EBL01 Reciprocal NIH EC90 ELISA titres 234
Figure 73: EBL01 Neutralising antibody titres 234
Figure 74: EBL01 Antibody responses to Sudan virus GP 235
Figure 75: EBL01 Baseline ELISPOT responses 237
Figure 76: EBL01 D14 post-prime ELISPOT responses 238
Figure 77: EBL01 Group 1-3 Median T-cell responses all timepoints 238
Figure 78: EBL01 ICS following ChAd3-EBO Z 239
Figure 79: EBL01 ICS Expression of CD107a post-prime/post-boost 239
Figure 80: EBL01 ICS Total cytokine post-prime/post-boost 240
Figure 81: EBL01 Effect of prime-boost interval on anti-GP titres 242
Figure 82: EBL01 Effect of prime-boost interval on nAbs 243
Figure 83: EBL01 Effect of prime-boost interval on ELISPOT responses 243
Figure 84: EBL01 Anti-GP GMT time course by prime-boost interval group 244
Figure 85: EBL01 Anti-GP Individual responses, short interval boosting 244
Figure 86: EBL01 Effect of short interval boosting on anti-GP titres 245
Figure 87: EBL01 Effect of short interval boosting on nAb titres 245
Figure 88: EBL01 Effect of short interval boosting on ELISPOT responses 246
Figure 89: EBL01 Prime–boost interval & peak ELISPOT response 246
Figure 90: EBL01 Individual at ELISPOT responses at M+7 to summed GP pools 247
Figure 91: EBL01 Individual ELISPOT responses at M+28 to summed GP pools 247
Figure 92: EBL01 ICS responses with short interval boosting 248

List of Tables

Table 1: Clinical trials of ME-TRAP encoding vaccines by University of Oxford. 52
Table 2: Summary of the VRC Studies for Evaluation of Ebola Vaccines 66
Table 3: Formulation and presentation of RTS,S/AS01B 75
Table 4: Compensation rates 88
Table 5: Solicited AEs as collected on post vaccination diary cards 99
Table 6: Malarial Diagnosis for Primary Endpoint Evaluation 104
Table 7: Guidelines for assessing the relationship of vaccine to an AE 111
Table 8: Severity grading criterion for injection site AEs 111
Table 9: Severity grading criterion for AEs. 112
Table 10: Severity grading criteria for physical observations 112
Table 11: VAC055 Clinical Trial Design 119
Table 12: Demographics of all subjects enrolled in VAC055 122
Table 13: VAC055 Proportion of subjects completely protected 123
Table 14: VAC055 Secondary efficacy endpoints 126
Table 15: VAC055 Time to secondary efficacy endpoint 127
Table 16: VAC055 Parasite density endpoints 127
Table 17: VAC055 Tertiary endpoint 131
Table 18: VAC055 All Unsolicited AEs 136
Table 19: VAC055 Grade 3 Unsolicited AEs 136
Table 20: VAC055 Related Unsolicited AEs 136
Table 21: Comparison of peptide sequences in ME-TRAP & RTS,S 150
Table 22: VAC059 Clinical Trial Design 162
Table 23: VAC059 Demographics of all subjects enrolled 165
Table 24: VAC059 Proportion of subjects completely protected 166
Table 25: VAC059 analysis of Primary and Secondary efficacy endpoints 171
Table 26: VAC059 Secondary efficacy endpoints 171
Table 27: VAC059 Solicited adverse events comparison 176
Table 28: VAC059 All unsolicited AEs comparison 177
Table 29: VAC059 Grade 3 AEs comparison 177
Table 30: VAC059 Related unsolicited AEs comparison 177
Table 31: VAC059 Table of all laboratory adverse events 178
Table 32: EBL01 Study dose groups 211

Table 33: EBL01 Extension dose groups 212
Table 34: EBL01 Demographics of all subjects enrolled..... 216
Table 35: EBL01 Solicited adverse events following ChAd3-EBO Z..... 222
Table 36: EBL01 Unsolicited AE summary after ChAd3-EBO Z 223
Table 37: EBL01 Solicited adverse events following MVA-BN® Filo..... 225
Table 38: EBL01 Unsolicited reactogenicity summary following MVA-BN® Filo..... 226
Table 39: EBL01 All laboratory adverse events following ChAd3-EBO Z..... 227
Table 40: EBL01 All laboratory adverse events following MVA-BN® Filo..... 228
Table 41: VAC055 Schedule of clinical reviews pre CHMI Groups 1 & 2..... 261
Table 42: VAC055 Schedule of clinical reviews pre CHMI Groups 3 & 4..... 263
Table 43: VAC055 Schedule of Clinic Attendances Post CHMI 264
Table 44: VAC059 Schedule of clinical reviews pre CHMI for Groups 1-4..... 267
Table 45: VAC059 Schedule of clinical reviews for Group 5 & 6 pre CHMI..... 269
Table 46: VAC059 Schedule of Clinic Attendances Post CHMI 270
Table 47: EBL01 Schedule of attendances for unboosted subjects 272
Table 48: EBL01 Schedule of attendances for all boosted subjects..... 273
Table 49: EBL01 Schedule of attendances for Group 4 & 6 subjects 275
Table 50: Schedule of attendances for Group 5 & 7 subjects 276
Table 51: Median live CD3+ cells acquired per sample by trial 279
Table 52: TRAP peptide pool format Peptide sequences..... 281
Table 53: ME peptide pool format..... 282
Table 54: CSP Peptide sequences..... 283
Table 55: Antibodies used for flow cytometry..... 283
Table 56: VAC055 Solicited adverse events..... 295
Table 57: VAC055 Unsolicited AEs reported by Group 1 vaccine 1 297
Table 58: VAC055 Unsolicited AEs reported by Group 2 vaccine 1 297
Table 59: VAC055 Unsolicited AEs reported by Group 1 vaccine 2 299
Table 60: VAC055 Unsolicited AEs reported by Group 1 vaccine 3 300
Table 61: VAC055 Unsolicited AEs reported by Group 2 vaccine 2 301
Table 62: VAC055 Unsolicited AEs reported by Group 1 vaccine 4 301
Table 63: VAC055 Unsolicited AEs reported by Group 2 vaccine 3 302
Table 64: VAC055 Unsolicited AEs reported by Group 1 vaccine 5 303
Table 65: VAC055 All abnormal laboratory results pre CHMI 304
Table 66: VAC055 All abnormal laboratory results post CHMI..... 306

Table 67: VAC059 Solicited adverse events 308
Table 68: VAC059 Unsolicited adverse events full listing 317
Table 69: EBL01 All unsolicited AEs reported following ChAd3-EBO Z 321
Table 70: EBL01 Related unsolicited AEs following ChAd3-EBO Z 322
Table 71: EBL01 All unsolicited AEs reported following MVA-BN® Filo 324
Table 72: EBL01 Related unsolicited AEs following MVA-BN® Filo 326

List of Abbreviations Used	
Ab	Antibody
Ad26	Human adenovirus type 26
Ad5 or (AdHu5)	Human adenovirus type 5
AdHu*n*	Human adenovirus where *n* denotes the serotype
AE	Adverse Event
AR	Adverse reaction
AS*nn*	GSK proprietary Adjuvant System where *nn* distinguishes the adjuvant components
AUC	Area Under the Curve
BCG	Bacillus Calmette Guérin
BDBV	Bundibugyo virus
C-1	The day before controlled human malaria infection
C+*n*	Where *n* is the number of days elapsed since controlled numan malaria infection
C0	The day of controlled numan malaria infection
CBF	Clinical Biomanufacturing Facility
CEF	Chicken embryo fibroblasts
ChAd (also AdCh)	Chimpanzee adenovirus
ChAd3-EBO Z (also denoted cAd3 EBO)	Chimpanzee adenovirus type 3 encoding Zaire Ebolavirus glycoprotein
ChAd63 ME-TRAP	Chimpanzee adenovirus type 63 encoding thrombospondin related adhesion protein fused to a multiple epitope string
CHMI	Controlled human malaria infection
CI	Confidence interval as defined by the preceding number (eg. 95%)
CI	Chief Investigator
CL	Containment level
CS	Circumsporozoite protein
CTL	Cytotoxic T-Lymphocyte
CTRG	Clinical Trials and Research Governance Team of the University of Oxford
D*n*	Where n is the number of days elapsed since receipt of first trial vaccine
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
DSMB	Data Safety Monitoring Board
DTPw	Diphtheria, tetanus, whole cell pertussis vaccine
E.g.	Exempli gratia (for example)
EBOV (also denoted ZEBOV)	Zaire Ebola virus
EHF	Ebola haemorrhagic fever

ELISA	Enzyme Linked Immunosorbent Assays
ELISPOT	Enzyme-Linked Immunosorbent Spot assay
EPI	Expanded Programme on Immunisation
EU	ELISA Units
EVD	Ebolavirus disease
GCP	Good Clinical Practice
GMO	Genetically Modified Organisms
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titre
GP	Glycoprotein
GP	General practitioner
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEK 293	Human embryonic kidney cells 293
Hib	*Haemophilus influenzae* type B
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HTLV	Human T-lymphotrophic virus
i.e.	Id est (that is)
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	Flow cytometry with intracellular cytokine staining
IDT	Impfstoffwerk Dessau-Tormau
IFNγ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular
IMP	Investigational Medicinal Product
IQR	Interquartile range
IRS	Indoor Residual Spraying
ITN	Insecticide treated mosquito net
LPS	Lipopolysaccharide
LSC	Local safety committee
LSM	Local safety monitor
ME	Multiple epitopes
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	UK Medicines and Healthcare products Regulatory Agency
mL	Millilitre
MPAC	Malaria Policy Advisory Committee

MPL	Mono-phosphoryl lipid A
MVA	Modified Vaccinia virus Ankara
MVA-BN Filo	Modified vaccinia virus Ankara encoding multiple filovirus proteins
nAb	Neutralising Antibody
NANP	4 amino acid repeat section of the CS protein (Asparagine, Alanine, Asparagine, Proline)
NHP	Nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	US National Institutes of Health
NMRI	Naval Medical Research Institute
OPV	Oral polio vaccine
P.	*Plasmodium*
p/mL	Parasites per mL
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase chain reaction
PfCSP	*Plasmodium falciparum* circumsporozoite protein
PfSPZ	cryopreserved whole sporozoite vaccine
pfu	Plaque forming units
QC	Quality Control
QP	Qualified person
qPCR	Quantitative polymerase chain reaction
QS21	Saponin molecule from Quillaja saponaria tree
rAd	Recombinant adenovirus
RBM	Roll Back Malaria Initiative
RC-1	The day before repeat controlled human malaria infection
RC+*n*	Where *n* is the number of days elapsed since repeat controlled human malaria infection
RC0	The day of repeat controlled human malaria infection
Ref.	Reference number or ID
RESTV	Reston virus
RR	Relative risk
RTS,S	B-cell repeats and T-cell epitopes fused to, and co-expressed with Hepatitis B surface antigen
SAE	Serious adverse event
SAGE	WHO's Strategic Advisory Group of Experts on Immunisation
SAR	Serious adverse reaction
SD	Standard Deviation
SFC	Spot Forming Cells
SUDV	Sudan virus

SUSAR	Suspected unexpected serious adverse reaction
TAFV	Tai-forest virus
TRAP	Thrombospondin-related adhesion protein
VE	Vaccine Efficacy
VHF	Viral Haemorrhagic Fever
vp	Virus particles
VPP	Vaccine research centre vaccine pilot plant
VRC	Vaccine research centre
VSV	Vesicular-stomatitis virus
WBC	White blood cell
WHO	World Health Organisation
WRAIR	Walter Reed Army Institute of Research
WT	Wild type
β HCG	beta-human chorionic gonadotrophin
ΔTM	Transmembrane deleted Ebola glycoprotein
µg	Micrograms

Chapter 1

Introduction

1. Chapter 1: Introduction

1.1. Rationale for Thesis

Efforts to develop a vaccine for *Plasmodium falciparum* malaria (*P. falciparum*) have been ongoing for decades. It has become apparent that a multi-faceted approach combining vaccines on different platforms, including viral vectors, may be beneficial in order to engage different components of the immune system. In 2014, an outbreak of a different tropical infection, Ebola, caused nearly 30 000 cases and killed over 11 000 people.[1] When the outbreak began, there was no vaccine available, or even in advanced stages of development. Lessons learned from research into viral vectored vaccines targeting malaria could be applied to diseases caused by outbreak pathogens, such as Ebolavirus, to quickly develop vaccines on a platform suitable for rapid, large scale manufacture, thereby reducing the impact of potentially devastating future outbreaks.

1.2. Diseases of the Tropics

Tropical diseases are those that are prevalent either solely or principally in the tropics. Frequently, these are infectious diseases that require specific climate conditions (e.g. hot and humid environments, seasonal rains etc.) or ecological factors such as animal reservoirs or insect vectors in order to thrive. Many other factors such as regional socioeconomic conditions, environmental change,[2,3] migration patterns, and population density play a key role in the distribution of these diseases.

1.3. Vaccines for Tropical Diseases

A number of different vaccines currently exist for infections that are more prevalent in the tropics. They are a mixture of protein subunit, inactivated and live attenuated vaccines. Some vaccines, such as rabies are given primarily as travel vaccines, either pre- or post-exposure, whilst others, such as BCG and yellow fever have been incorporated into the Expanded Programme on Immunisation (EPI) schedules for many tropical countries. There are numerous infectious diseases for which no licensed vaccines currently exist, and the reasons behind this are many and varied. Diseases such as malaria and HIV are leading contributors to the global health burden in terms of morbidity, mortality and economics, but intense efforts to develop vaccines are yet to yield suitable candidates for licensure and widespread deployment. This is, in part, due to the complex immunology and pathogenesis of the underlying disease [4-6]. Alternatively, other diseases such as the filoviral haemorrhagic fevers are still relatively uncommon diseases in humans, and consequently, there is a relative paucity of research focussing on treatments and vaccines. The work presented in this thesis is of clinical trials of experimental vaccines for *P. falciparum* malaria, and also for Ebolavirus, which is a cause of viral haemorrhagic fever (VHF).

1.4. Malaria

Malaria is a complex, mosquito-borne infection caused by a protozoan parasite. It is one of the leading causes of mortality in low income countries[7] and there is urgent

need for a vaccine. Five species of Plasmodium are known to cause disease in humans; *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, with the majority of deaths attributable to *P. falciparum*.[8] There is evidence to suggest that the epidemiology of *P. falciparum* malaria may be changing across certain parts of Africa[9], but the worldwide burden of disease remains a major public health problem with an estimated 214 million cases and 438,000 deaths worldwide in 2015.[8] 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[9].

In recent years, efforts to reduce the global health impact of malaria have resulted in a considerable degree of success, with a decline in absolute mortality of 48% from 839,000 deaths in 2000 to 438,000 in 2015, and an estimated decrease in malaria mortality rate, over this same period, of approximately 60%. Amongst key contributors to this reduction in mortality are:

- Access to insecticide-treated mosquito nets (ITNs)
    - Increase in <5s sleeping under ITNs from <2% in 2000 to 68% in 2015[8]
- Indoor residual spraying (IRS)
    - Proportion of population protected peaked at 5.7% in 2010
    - Estimated 3.4% in 2014[8]
- Chemoprophylaxis in pregnancy – 52% received at least 1 dose[8]
- Chemoprevention in children – 15 countries have recommended seasonal prevention
- Increase in diagnostic testing

- Access to treatment

1.4.1. Epidemiology and Global Burden of Disease

Globally, malaria exhibits a variety of epidemiological patterns. This is due, in part to the numerous species of female mosquito that have the capability to transmit disease. Malaria transmitting mosquitoes all belong to the *Anopheles* genus, but different species exhibit different behavioural and biological characteristics, which can lead to varying epidemiology. Key determinants of malaria epidemiology include the lifespan and biting habits of the female mosquitoes, and the vector density.

1.4.2. Malaria Transmission Dynamics

Effective malaria vector control and preventative measures would not be possible without a detailed understanding of the dynamics of malaria transmission. In the late 19th Century, Patrick Manson was the first to demonstrate that mosquitoes transmitted blood-borne pathogens, and shortly after, through collaboration with Manson, Ronald Ross demonstrated that mosquitoes were responsible for the transmission of malaria.[10] Ross developed several theoretical mathematical models of malaria transmission, which was greatly developed by George Macdonald in the 1950s, culminating in several different models, and modeling styles that are commonly called “Ross-Macdonald” models. A Ross-Macdonald model is based on a simplified version of the pathogen life-cycle that has been quantitively and mathematically described in four steps.

1. During a blood meal, the pathogen is passed from a mosquito to a vertebrate host.

2. In the vertebrate host, the pathogen multiplies, reaching sufficiently high densities to infect a new mosquito.

3. A susceptible mosquito takes a blood meal from the infected vertebrate host and imbibe the pathogen.

4. The pathogen then develops in the mosquito to the point where it is anatomically placed to be transmitted to a new vertebrate host during a blood meal.

There are numerous models with different formulae for each step, but there is a unifying set of assumptions regarding transmission dynamics and host factors that are common to each model. Further characterisation of malaria transmission, has been described direct relationships with the vector density, the square of the number of human bites per day per mosquito, and the daily survival rate of the mosquito to the power of 10.[11]

1.4.3. Life Cycle and Pathogenesis

Human malaria occurs following a bite from a female anopheline mosquito, during which sporozoites are injected from the salivary glands of the mosquito into the bloodstream of the host. Over a period of minutes, the sporozoites travel from the inoculation site to the liver, where they invade hepatocytes and the liver stage of infection begins. The asymptomatic liver stage lasts for approximately 6-16 days, and is generally shorter in *P. falciparum* infection than infection with other species. During this period, the sporozoites grow and divide into haploid forms called merozoites. Infected hepatocytes mature into hepatic schizonts, and can contain

tens of thousands of merozoites each. Some species of malaria (*P. ovale, P. vivax*) also produce hypnozoites in the liver that can remain dormant for long periods, and can cause relapse in disease weeks or months later.

Upon rupture of hepatic schizonts, the merozoites enter the bloodstream, beginning the blood stage of malaria infection, and commencing a cycle of erythrocyte invasion known as asexual replication. Once inside the red blood cells, merozoites reproduce producing roughly 12-16 daughter merozoites per schizont. The schizonts then rupture in near synchrony releasing the merozoites into the bloodstream and allowing the cycle of erythrocyte invasion, merozoite multiplication and schizont rupture to begin again. This cycle of invasion and cell rupture takes 1-3 days depending on the species of malaria and can lead to thousands of parasite-infected erythrocytes in the host bloodstream.

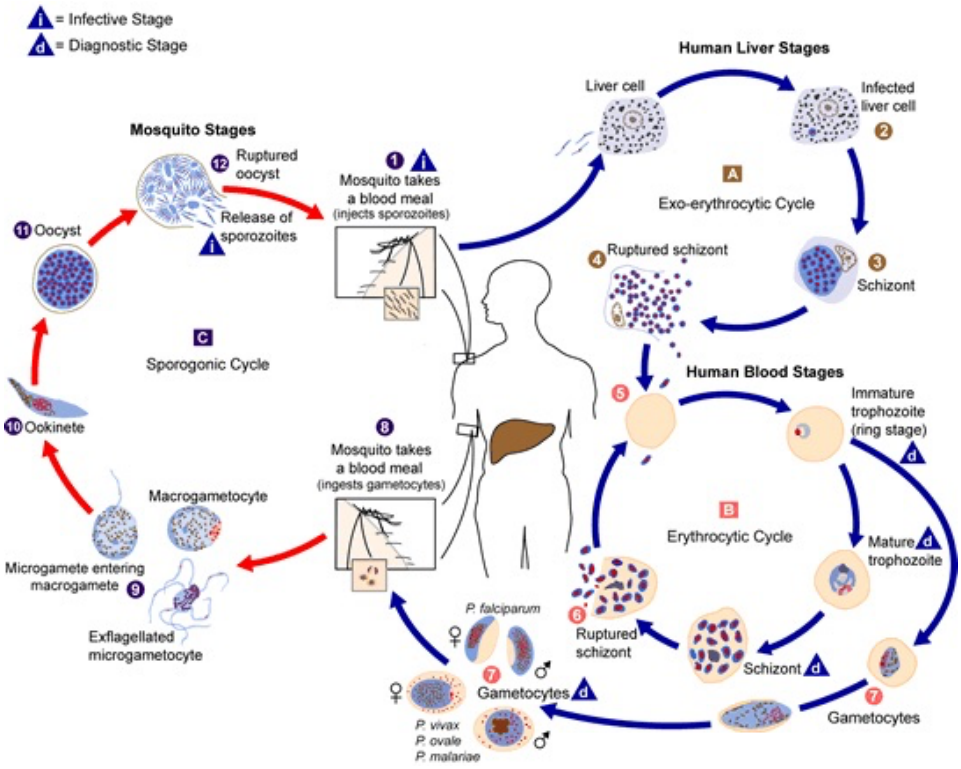


Figure 1: Malaria parasite life cycle. Adapted from www.cdc.gov/malaria/about/biology/

During the blood stage of infection, some of the merozoite-infected erythrocytes leave the cycle of asexual replication to develop into male and female gametocytes (termed microgametocytes and macrogametocytes respectively), the sexual forms of the parasite. The gametocytes are ingested by an *Anopheles spp.* mosquito when it bites an infected human and takes a blood meal. The infected erythrocytes rupture in the midgut of the mosquito releasing the parasites. The gametocytes then develop further into mature sexual forms called gametes and diploid zygotes are formed when the microgametes penetrate the macrogametes. Zygotes develop into ookinetes that invade the mosquito midgut where they develop into oocysts. The oocysts grow and divide producing thousands of haploid sporozoites, and after a period of 8-15 days, they burst releasing the sporozoites. The sporozoites make their way to the mosquito's salivary glands where they can be inoculated into a new human host when the mosquito takes a blood meal.

The pre-erythrocytic stage of malaria infection is asymptomatic. Symptoms begin only after the hepatic schizonts rupture releasing merozoites into the blood stream. In addition to the release of newly formed merozoites, numerous known, and unknown substances are released upon schizont rupture, such as free haem, haemozoin and remnants of cell membranes. These products of erythrocyte lysis trigger a variety of processes that lead to the initial symptoms of malaria, and the classic clinical manifestations of paroxysmal fevers and chills, are associated with the synchronous rupture of the schizonts. Macrophages phagocytose the remnants of cell membrane and hemozoin, in turn stimulating activation of the immune

cascade,[12,13] and free haem can stimulate endothelial activation, in some cases leading to endothelial cell damage.[14] The process of merozoite maturation, from erythrocyte invasion to schizont rupture occurs over 24 hours (*P. knowlesi*), 48 hours (*P. vivax, P. ovale, P. falciparum*), or 72 hours (*P. malariae*), and paroxysms occur following each cycle.

1.4.4. *Plasmodium Falciparum* Malaria

Falciparum malaria is caused by the species *P. falciparum*. It is both the most prevalent species to cause human disease, and the most deadly.[8] If managed appropriately, and in a timely fashion, falciparum malaria can be treated successfully, and the disease follows a generally mild course. If falciparum malaria is not identified and treated early enough, however, severe complications can develop and the disease becomes life threatening. Young children and non-immune adults are at highest risk. *P. falciparum* differs from other species of malaria due to the distinctive property of red cell sequestration. As the merozoites mature from rings to trophozoites within erythrocytes they alter the host cell membranes with parasite encoded proteins that form cytoadherent protrusions. These protrusions bind to endothelial receptors leading to sequestration of infected erythrocytes within the small vessels. This then leads to endothelial barrier breakdown, partial blood flow obstruction, and inflammation.[15,16] Any organ can be affected by sequestration, with the most severe clinical consequence of sequestration being cerebral malaria.

1.4.5. Attempts at global control and potential elimination of malaria

In 1998, the World Health Organisation (WHO) launched the Roll Back Malaria Initiative (RBM). RBM was a health sector-wide partnership aimed at combatting the impact of malaria at global, regional, country and local levels.[17] Through the greatly increased investment of technical assistance and financial resources, malaria control has intensified in the 18 years since the RBM initiative was launched, and the elimination of malaria is an increasingly realistic prospect in a number of countries. In 2000, only 13 countries were estimated to have fewer than 1000 annual malaria cases, whereas by 2015, this had increased to 33 countries.[8] Three countries reported fewer than 10 cases in 2014, and in the same year, 16 countries reported no indigenous cases. Despite the apparent success of the RBM initiative, there remain many ongoing challenges, which are summarised by the WHO as follows:

- Slower declines in malaria in high-burden countries: Although malaria is considered endemic in 109 countries, by 2015, it was estimated that only 15 countries account for 80% of all malaria cases and 78% of malaria related deaths, with countries in sub-Saharan Africa dominating the global burden of disease. Countries that reported the highest malaria incidence and mortality in 2000 also report the slowest decline in disease burden since the launch of the RBM initiative.
- Gaps in intervention coverage: Millions of people, particularly in the poorest counties with the highest disease burden are still not able to access drugs, ITNs or IRS.

- Weaknesses in health systems in countries with the greatest malaria burden: Many of the countries with the highest numbers of malaria cases are also amongst the lowest gross national incomes and total domestic government spending per capita than the countries with fewer cases. This, in turn limits the ability of malaria endemic countries to strengthen health systems and improve access to treatment and preventative measures.
- Economic burden of malaria on health systems: Given that malaria burden is highest in some of the world's poorest regions, the cost of malaria treatment is disproportionately high in the most resource-limited countries.
- Insecticide resistance: ITNs and IRS will remain effective only as long as the insecticides are effective, and the emergence of insecticide resistance is a concern. Since 2010, 60 out of 78 countries collecting data reported resistance to at least one insecticide in one malaria mosquito population, and 49 out of 78 detected resistance to two or more insecticide classes.
- Antimalarial drug resistance: Artemesenin resistance has now been reported in 5 countries in Southeast Asia. There is considerable concern with regard to the potential spread of artemsenin resistance to the countries with the highest disease burden, particularly those in sub-Saharan Africa.[8]

Amongst many goals outlined in the RBM initiative was a commitment to provide and facilitate effective investment in new medicines, vaccines and other tools to reduce malaria transmission, and disease burden. Vaccines would be a vital tool in the quest to eliminate malaria, and in 2006, the Malaria Vaccine Technology Roadmap was published, with a subsequent revision in 2013. The revised strategic goals were to have licensed vaccines by the year 2030 targeting *P. falciparum* and *P.*

vivax that produce a protective efficacy of at least 75% lasting 2 years or more, and requiring booster doses no more frequently than annually. An additional goal was the development of vaccines suitable for mass administration that reduce the transmission of malaria.[18] In addition to the development of vaccines, novel strategies, such as the use of CRISPR Cas9-mediated genetic engineering to create transgenic mosquitoes are being evaluated.[19] These mosquitoes would carry antipathogen effector genes targeting human malaria parasites, thereby rendering them incapable of transmitting malaria to humans.

1.4.6. History, and Current Status of Malaria Vaccine Development

At present, there is no licensed vaccine against malaria. A small number of candidate vaccines have shown partial efficacy against experimental and natural human infection, but efficacy is limited. Efforts to develop effective vaccines are complicated by the complex immunology of malaria infection, and the lack of a reliable natural model of complete immunity. Concerted efforts in malaria vaccine research began in the 1930s following on from the Sergent brothers attempts to induce immunity in canaries in 1921.[20] The early focus was on attenuated or inactivated whole parasites, but encouraging protective results in animal models were not achieved until adjuvants were added to inactivated parasites in 1945.[21] Partial efficacy in humans was demonstrated in 1975 through the use of attenuated sporozoites inoculated by mosquito bite,[22] but results were transient and this approach was considered impractical for large scale vaccine deployment. By the 1980s, focus had shifted toward synthetic peptide constructs with or without

adjuvant, and more recently, DNA vaccines, viral vectored vaccines and injectable, frozen whole irradiated sporozoites have all been evaluated with varying degrees of success.

The candidate vaccine currently the most advanced in clinical trials is the recombinant protein in adjuvant vaccine, RTS,S/AS01. RTS,S targets the circumsporozoite protein (CS), which is expressed by the *P. falciparum* sporozoite at the pre-erythrocytic stage (Figure 1).

1.4.7. The Pre-Erythrocytic Stage of Malaria Infection as a Vaccine Target

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 parasitisation of red blood cells and initiation of the blood stage of infection. If a vaccine can induce sufficient anti-sporozoite immunity, primarily through humoral mechanisms, then the parasites can be targeted and destroyed 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. Research suggests that, in isolation the RTS,S vaccine targeting the pre-erythrocytic stage antigen, CS, and vaccines targeting the liver stage antigen thrombospondin related adhesion protein (TRAP) do not delay the initial emergence of parasites in to the blood, nor the rate of parasite multiplication in the blood, but rather reduce the size of this initial inoculum[23]. A delay to patent blood stage infection in persons receiving

these vaccines reflects a reduced liver-to-blood inoculum. The efficacy of these pre-erythrocytic vaccine strategies can be assessed experimentally by subjecting volunteers to inoculation with *P. falciparum* sporozoites by the bite of infected mosquitoes. Complete protection against blood-stage infection, or a delay in the time to patent blood stage infection in vaccinees compared to controls, is interpreted to better understand mechanisms of vaccine-induced protection.

1.4.8. RTS,S/AS01B

RTS,S targets the CS protein, 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.[24] The RTS,S antigen is formed of two proteins, RTS and S, that intracellularly and spontaneously assemble into mixed polymeric particulate structures. RTS,S is produced in *Saccharomyces cerevisiae*, genetically modified to contain the coding sequences of the RTS and S proteins. The RTS hybrid polypeptide consists of a repeat region of 4 amino acids (NANP) and the entire C-terminal flanking region of the *P. falciparum* NF54 strain CS antigen, fused to the amino terminal end of the wild-type Hepatitis B virus surface protein (HBsAg). The name, RTS,S denotes the repeat region (R), T-cell epitopes in the flanking regions (T) and HBsAg (S).

The AS01 family of Adjuvant Systems (including AS01B) contains 2 immunostimulants formulated with liposomes. The monophosphoryl lipid A (MPL) molecule consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the Gram negative bacterium *Salmonella minnesota*. QS21 is a natural

saponin molecule purified from the bark of the South American tree, *Quillaja saponaria*.

Historically, RTS,S/AS01B has demonstrated a vaccine efficacy (VE) of approximately 50%, against controlled human malaria infection.[25] 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.[26,27] Vaccine efficacy wanes over time, but can be enhanced by a fourth dose.[28] 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,[25] 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[29,30] suggesting that anti-CS antibodies may also be associated with the duration of protection. A number of factors, including age at vaccination, HIV status and high baseline anti-CS antibody titres impact anti-CS antibody titres after vaccination with RTS,S.[31]

1.4.8.1. RTS,S Evaluation in the Controlled Human Malaria Infection Model

The controlled human malaria infection (CHMI) model has been instrumental in identifying candidate approaches worthy of further evaluation in conditions of natural transmission, and has been found to be a good predictor of field efficacy. Early clinical development of the RTS,S malaria vaccine was initiated in studies in malaria-naïve adults in collaboration with the Walter Reed Army Institute of

Research (WRAIR) in which efficacy, safety and immunogenicity of the RTS,S/AS02$_A$ vaccine formulation were demonstrated.[24,32]

In the first Phase I/IIa study, RTS,S formulated either in an oil-in-water emulsion (AS03), or with aluminium salts + MPL (AS04), or in oil-in-water emulsion + MPL + QS-21 (AS02), was tested in the CHMI model.[24] Non-immune healthy adults received RTS,S/AS04 (N=14), RTS,S/AS03 (N=15) or RTS,S/AS02 (N=17). Following CHMI, of the 7 subjects who received RTS,S/AS02A, 1 subject developed malaria, representing an estimated vaccine efficacy (VE) of 86% (RR=0.14; 95% CI: 0.02-0.88, p<0.005). All subjects in the control group became parasitaemic following challenge. High anti-CS and anti-HBsAg antibody titres were observed in recipients of RTS,S/AS02A.

Further studies confirmed that 2 or 3 doses of RTS,S/AS02A consistently conferred complete protection in around 35% to 50% of subjects who underwent CHMI[25,32-34]. Across 3 studies, 3 doses of RTS,S/AS02$_A$ demonstrated protection of 37.5% (95% CI: 26.5 – 46.8) of healthy non-immune volunteers against homologous CHMI.[25,32,33]

In parallel to the continued evaluation of RTS,S/AS02 in endemic countries, the RTS,S antigen was combined with the Adjuvant System of the AS01 family in order to improve immunogenicity and potentially efficacy. In a CHMI trial, the safety and reactogenicity of RTS,S/AS01B was comparable to that of RTS,S/AS02A,[25] and a trend towards improved VE against infection with RTS,S/AS01B compared to RTS,S/AS02A was observed (50.5% [95% CI: 32.9, 67.1] vs 31.8% [95% CI: 17.6, 47.6]).

1.4.8.2. RTS,S Phase II Paediatric Evaluation

Vaccine efficacy (VE) of the earlier formulation, RTS,S/AS02, against malaria and severe malaria, was demonstrated in a large trial that enrolled 2022 children aged 1 to 4 years from an area of high malaria transmission (Mozambique). Over the first 18 months of follow-up, efficacy against first clinical malaria episode (defined as *P. falciparum* parasitemia >2500/µL and fever >37.5°C) was 35.3% (95% CI: 21.6, 46.6; p<0.0001), and against severe malaria disease was 48.6% (95% CI: 12.3, 71.0; p=0.02).[35] The clinical benefit of vaccination persisted for 3.5 years after vaccination; VE for protection against all clinical episodes was 25.6% (95% CI: 11.9, 37.1; p<0.001) over 42 months, with no rebound of malaria cases in the RTS,S/AS02 vaccinated group.[36] Throughout the follow-up period, cross-sectional parasitemia surveys showed a 30-35% decrease in prevalence of parasitemia in the RTS,S/AS02 group.[36]

Clinical malaria efficacy of RTS,S/AS01 has been assessed in a proof-of-concept study conducted in Kenya and Tanzania. In this trial VE against malaria disease observed in children 5 to 17 months of age vaccinated with 3 doses of the RTS,S/AS01 candidate vaccine was 52.9% (95% CI: 28.1, 69.1; p<0.001: mean follow-up time of 8 months).[37] Over 1 year follow-up after completion of immunisation, efficacy against first or only episodes of malaria disease was 39.2% (95% CI: 19.5, 54.1; p=0.0005) and was 45.8% (95% CI: 24.1, 61.3; p=0.0004) when children were followed up to the end of the extended follow up period (mean follow-up time of 15.4 months, 1 site only).

In addition, VE against *P. falciparum* infection (65.9% [95% CI: 42.6, 79.8]; p>0.0001) and against clinical malaria (65.8% [95% CI: 25.3, 84.4]; p=0.007) has been demonstrated with RTS,S/AS02 in infants from 6 weeks of age followed for 3 months.[38] A similar efficacy against infection (65.2% [95% CI: 20.7, 84.7]; p=0.02) was shown after 6 months of follow-up when RTS,S/AS02 was co-administered with DTPw/Hib and OPV vaccines according to the EPI schedule.[39]

Both formulations of the RTS,S/AS vaccine have been shown to have an acceptable clinical safety profile when administered to infants and children in sub-Saharan Africa. In total, more than 8000 doses of RTS,S/AS have been administered to over 3000 infants and children. The reactogenicity (solicited local and general symptoms) of the RTS,S/AS01 vaccine was comparable to that induced by DTPw combination vaccines.[38]

1.4.8.3. RTS,S/AS01 Phase III Evaluation

The pivotal Malaria-055 Phase III efficacy study was a large double-blinded, randomised, controlled multicentre study carried out in 11 centres in 7 African countries, across diverse malaria transmission settings. This study was conducted in two age groups of children; those aged 6 to 12 weeks (n=6537) and those aged 5 to 17 months (n=8922) at enrolment. The study evaluated three study groups in each age category: three doses of RTS,S/AS01 administered at 0, 1, 2-months plus a booster dose at 20 months (R3R Group); three doses of RTS,S/AS01 administered at 0, 1, 2-months without a booster dose of a comparator vaccine at 20 months (R3C Group), and a non-malaria comparator vaccine control group with vaccine

administered at 0, 1, 2 and 20 months. The co-primary end points of the trial were met.[40,41] From month 0 until the study end, VE against clinical malaria in the R3R group was 36·3% (95% CI 31·8–40·5), and 28·3% (95% CI 23·3–32·9) in the R3C group. VE against severe malaria was in the R3R group was 32·2% (95% CI 13·7 to 46·9) and 1·1% (95% CI –23·0 to 20·5) in the R3C group. The frequency of serious adverse events (SAEs) was balanced between groups, however, meningitis was reported as a SAE in 21 children in the groups that received RTS,S/AS01, and only 1 in the C3C group.[28]

In October 2015, the WHO's Strategic Advisory Group of Experts on Immunisation (SAGE) and the Malaria Policy Advisory Committee (MPAC) jointly recommended pilot projects to evaluate the best future course of action for RTS,S/AS01. SAGE/MPAC recommended the initial introduction of 4 doses of the malaria vaccine in 3-5 distinct epidemiological settings in sub Saharan Africa, at subnational level, as pilot implementation projects.

The main purposes of these would be to assess:

- The feasibility of administering 4 doses of RTS,S in routine vaccination programmes
- The impact of the vaccine on child mortality

Further information will also be provided on the safety of the vaccine to evaluate the risk of rare adverse events such as meningitis.[42]

1.4.8.4. Fractional Dose Boosting with RTS,S/AS01B

In 1997, during the first CHMI trial of RTS,S, reactogenicity concerns after the second dose of vaccine led to a reduction in the third dose in 2 of the study groups. One group received a regimen consisting of 2 standard doses of RTS,S/AS02 at 0 and 1 month, and a third dose at month 7 which was 1/5th of the standard dose. Following CHMI, 6/7 subjects remained protected, resulting in a vaccine efficacy of 86% [95% CI: 0·02, 0·88], p<0·005).[24] The beneficial effect of a fractional third dose on VE was further demonstrated in a recent CHMI trial, in which subjects received a 0, 1, 7-month regimen consisting of 2 standard doses of RTS,S/AS01B followed by a fractional (1/5th) third dose.[43] Following CHMI, 26/30 subjects were protected against malaria (VE 86.7% [95%CI 66.8-94.6] compared with 10/16 in the standard 0, 1, 2-month full dose group (VE 62.5 [95% CI 29.4-80.1); p=0.040). Fractional dose boosting with RTS,S/AS01B has not previously been assessed in a 0, 1, 2-month vaccination schedule.

1.4.9. Viral Vector Vaccines

In recent years, there has been interest in using viruses as vectors to deliver genetic material into cells for use in a variety of applications, including basic science, gene therapy[44] and vaccination.[45] Viral vector vaccines share some common principles with DNA vaccines. Following administration, viral vectors carry genetic material into host cells for production of antigenic proteins that have been specifically designed to elicit a range of immune responses, including humoral and cell mediated immunity. The viral vector vaccine generally consists of live attenuated virus that has been

genetically modified to carry DNA encoding protein antigens from an unrelated organism, against which protective immunity is desired.

One advantage that viral vectors have over DNA vaccination is the ability to actively invade host cells, thereby further activating the immune system like an adjuvant.

Some viral vectors are being used in a vaccination schedules known as heterologous prime-boost regimens. In these vaccination schedules, one viral vector containing a genetic insert for a specific antigen is given as a priming step, followed by a second vaccine booster, using the same antigenic insert, but a different viral vector. The heterologous prime-boost strategy aims to provide a stronger overall immune response, mainly by circumventing the problem of anti-vector immunity that may have been induced by the priming vaccine.

1.4.10. Adenovirus Vectored Vaccines

1.4.10.5. Human Adenoviruses

Adenoviruses are non-enveloped, double stranded DNA viruses with icosahedral capsids. They generally cause mild disease in humans but infection can occasionally be life threatening, particularly in immunocompromised individuals. They possess several attractive qualities as viral vectors and a favourable safety and efficacy profile of orally administered live human adenovirus serotype 4 (AdHu4) and 7 (AdHu7) was generated in a large US military cohort by mass vaccination campaigns in attempts to prevent respiratory disease.[46,47] Adenoviruses have a large and stable genome that allows for manipulation of the genetic DNA but also prevents deletion of inserted foreign genes. They induce both innate and adaptive immune responses,

and have a wide tissue tropism. After infection, the transferred information remains extra-chromosomal, thereby reducing the risk of insertional mutagenesis. Viral replication is dependent on genes at the E1 locus, and replication defective adenovirus can be engineered by deleting these genes. Replication defective adenovirus can then be easily propagated with good yields in cell lines expressing E1 from human adenovirus serotype 5 (AdHu5) such as human embryonic kidney cells 293 (HEK 293).[48]

Although human adenoviruses have been evaluated as vaccine vectors for a number of conditions, naturally acquired adenovirus infection in humans is ubiquitous. Pre-existing immune responses have the potential to modify the subsequent immune response to a vectored antigen. Therefore, the high background seroprevalence leads to concerns about anti-vector immunity, and this may be a limiting factor to their widespread use as vaccine vectors. Seroprevalance varies by geographical region, and estimates suggest that 45–80% of adults carry neutralising antibodies to AdHu5.[49] Vaccine responses to Immunisation with AdHu vectors is attenuated in animal models when they have previously been exposed to human adenoviruses. This is most likely due to the removal of virus particles by pre-existing neutralising antibodies.[50-52] The presence of pre-existing anti-vector AdHu immunity does not seem to negatively impact on reactogenicity of the vectors.[53] In the large-scale "STEP" clinical trial of an AdHu5 based HIV-1 vaccine, the vaccines showed a lack of efficacy and tended to increase the risk of HIV-1 infection in vaccine recipients who had pre-existing neutralizing antibodies to AdHu5.[54] Furthermore, using AdHu5 encoding HIV-1 gag in a homologous prime boost strategy did not improve the peak

post prime levels of gag specific lymphocytes. This was most likely due to anti-vector immunity induced by the priming vaccination.[55]

1.4.10.6. Simian adenoviruses

The high seroprevalence to human adenoviruses has led to the evaluation of non-human species-specific adenoviruses as vectors. Simian adenoviruses have very similar hexon protein structures to those of human adenoviruses.[56] For example the chimpanzee adenovirus type 63 (ChAd63) hexon is highly homologous to the hexon of AdHu4. As with human adenoviruses, deletion of the E1 locus in chimpanzee adenoviruses will render viruses replication deficient and allow transcomplementation on an E1 AdHu5 complementing cell line.[57] The risk of homologous recombination leading to production of replication competent virus is limited by the lack of sequence homology between AdHu5 and chimpanzee adenoviruses at the E1 flanking region.[58]

Simian adenoviruses are not known to cause pathology or disease in humans and the prevalence of antibodies to chimpanzee origin adenoviruses is less than 5% in humans residing in the US.[59] Seroprevalence is higher in parts of Africa where chimpanzees reside, but still below that of AdHu5. An epidemiological study in Kenya showed that 23% of children aged 1-6 years had high neutralising antibodies titres to AdHu5, whilst only 4% had high-titre neutralising antibodies to ChAd63. Immunity to both vectors was age-dependent.[60] A recent survey of 100 infants and children living in Banfora, Burkina Faso, has shown a 3% seroprevalence of neutralising antibodies to ChAd63[61] (Malaria Vectored Vaccine Consortium,

www.euvaccine.eu/portfolio/project-index/mvvc, unpublished data). Early mouse studies showed that in comparison to AdHu5 and poxvirus, ChAd68 was very effective at generating a transgene product specific $CD8^+$ T cell response.[62] In the same study, pre-exposure to AdHu5 diminished any protection offered by vaccination with AdHu5, with only minimal cross-reactivity when vaccinated with ChAd68. This encouraging finding suggests that pre-existing neutralising antibodies to human adenoviruses should not reduce the potency of the immune response generated to chimpanzee adenovirus vectored vaccines.

1.4.11. MVA Vectored Vaccines

Vaccinia virus is a large, complex, enveloped virus belonging to the poxvirus family. It has a linear, double-stranded DNA genome of approximately 190 kb in length, which encodes for around 250 genes. Vaccinia was identified as a candidate vaccine vector following the worldwide eradication of smallpox using vaccinia vaccination. The development of several attenuated strains of vaccinia was initiated due to the small but significant risk to both researchers and vaccine recipients of conventional replication-competent vaccinia virus. Modified vaccinia Ankara (MVA) is a vaccine vector that has been shown to be both safe and immunogenic. MVA was originally derived from the Turkish vaccinia strain Ankara following over 500 blind serial passages in primary chicken embryo fibroblasts (CEF cells). This process results in host-range restriction, and marked attenuation compared to other vaccinia viruses. During the passaging through CEFs, vaccinia virus loses approximately 15% of its genome, but the genome remains stable.[63] It is differentiated from the parental Ankara genome by 6 major genomic deletions and is severely compromised in its

ability to replicate in mammalian cells, such that no replication in non-transformed mammalian cells has been documented. MVA also showed no cytopathic effect or plaque formation in cells of human origin.

In addition to malaria and haemorrhagic fever virus vaccines, MVA is currently in development as a vaccine vector for multiple diseases including HIV,[64,65] tuberculosis,[66] Hepatitis C,[67] influenza[68] and several malignant diseases.[69,70] MVA has been shown to be safe in both animals[71] and humans including in some high-risk groups.[63,72] It has been shown to be non-lethal in irradiated mice, and did not elicit severe morbidity, even when injected into the brain.[63] Evaluation in heavily immunocompromised Simian Immunodeficiency Virus (SIV) infected macaques, and mice with severe combined immunodeficiency (SCID) has provided supportive evidence to the concept of safe administration of MVA vectors in immunocompromised humans.[73] Local and systemic adverse events from MVA vaccination in humans are predominantly mild in severity and self-limiting. MVA was licensed in Germany until the end of compulsory smallpox vaccination in 1980[71] and was included in the official immunisation schedule.[74] Viral replication of MVA is blocked late during infection of cells, but the synthesis of both viral and recombinant protein synthesis is allowed to progress unimpaired.

MVA vectored vaccines induce excellent boosting immune responses to antigens when given in a heterologous prime-boost sequence after a prime vaccine that utilises a different vector.[72] Intramuscular administration is currently the favoured route, as when compared with intradermal administration, clinical studies with MVA

ME-TRAP have shown it to be associated with fewer and shorter lived local AEs and no significant reduction in immunogenicity.[75]

1.4.12. ME-TRAP

Vectored vaccine strategies using the ME-TRAP recombinant insert have been in clinical development since 1999. ME-TRAP is a recombinant 2398 base-pair DNA insert which encodes for a single polypeptide of 789 amino acids[76,77] containing multiple epitopes (ME) and the *P. falciparum* pre-erythrocytic thrombospondin-related adhesion (or anonymous) protein (TRAP). ME is a string of 20 epitopes, mainly $CD8^+$ T cell epitopes, from *P. falciparum* pre-erythrocytic antigens. The individual cytotoxic T-lymphocytes (CTL) epitopes which constitute the ME part of ME-TRAP are recognized by a number of common human leukocyte antigen (HLA) types, and they represent a variety of potentially protective target antigens. They are included to increase the likelihood of vaccine induced protective immunity in vaccine recipients.[78] The ME string is fused to the entire sequence of the T9/96 strain of *P. falciparum* TRAP. TRAP is a well characterized and abundant pre-erythrocytic stage *P. falciparum* antigen. Delivery of ME-TRAP as a recombinant insert in the inactivated viral vectors, Chimpanzee adenovirus serotype 63 (ChAd63) and modified vaccinia Ankara (MVA), administered eight weeks apart in a heterologous prime-boost sequence has demonstrated efficacy, either when given alone or in combination with viral vectors encoding for other malaria antigens in CHMI trials conducted by the Jenner Institute at the CCVTM in Oxford. This was demonstrated

first in the MAL034[79] clinical trial (Figure 2) and again in the VAC045[80] (Figure 3) and VAC052 (Ewer/Payne/Rampling et al. in preparation) clinical trials.

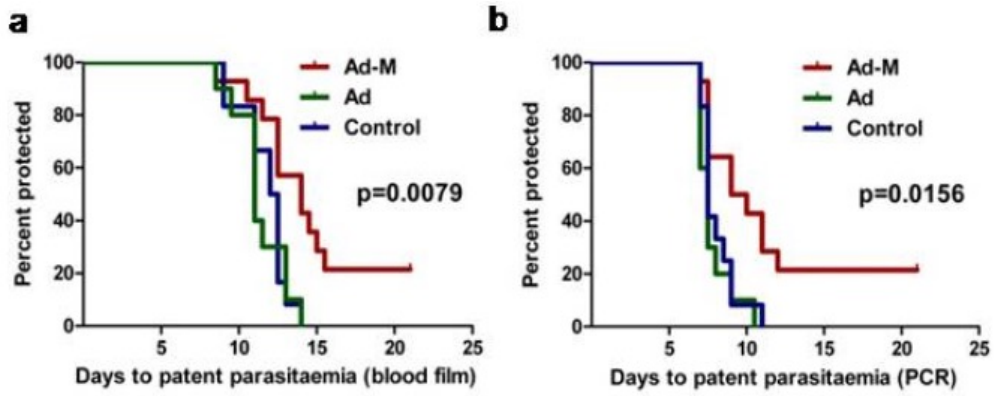


Figure 2: MAL034: Kaplan-Meier log rank comparison of days to positive blood film (a) and positive PCR (>20 parasites/ml) (b) following heterologous sporozoite challenge with 3D7 *P. falciparum*. Ad-M (red line): 14 volunteers who underwent malaria challenge after prime boost vaccination with ChAd63 ME-TRAP 5 x 10^{10} vp IM and MVA ME-TRAP 2 x 10^{8} pfu ID. Ad (green line): 10 volunteers who underwent malaria challenge after vaccination with ChAd63 ME-TRAP alone. Control (blue line): fourteen control volunteers. Fifty seven percent of ChAd63-MVA ME-TRAP vaccinees demonstrated clinical efficacy: 3/14 (21%) volunteers demonstrated sterile protection, and 5/14 (36%) volunteers demonstrated partial protection (an increased time to clinical malaria). Kaplan-Meier survival analysis demonstrated significant delay in time to patent parasitaemia in the prime-boost vaccinees compared to the control group as measured by blood film microscopy (p = 0.008, log rank test,) or a real time quantitative PCR assay[16] (to > 20 parasites/ml, p = 0.016, log rank test). On re-challenge 8 months later, all 3 sterilely protected ChAd63-MVA ME-TRAP volunteers demonstrated evidence of persisting efficacy, with 1 volunteer demonstrating sterile protection and the other two delay to patency (data not shown).

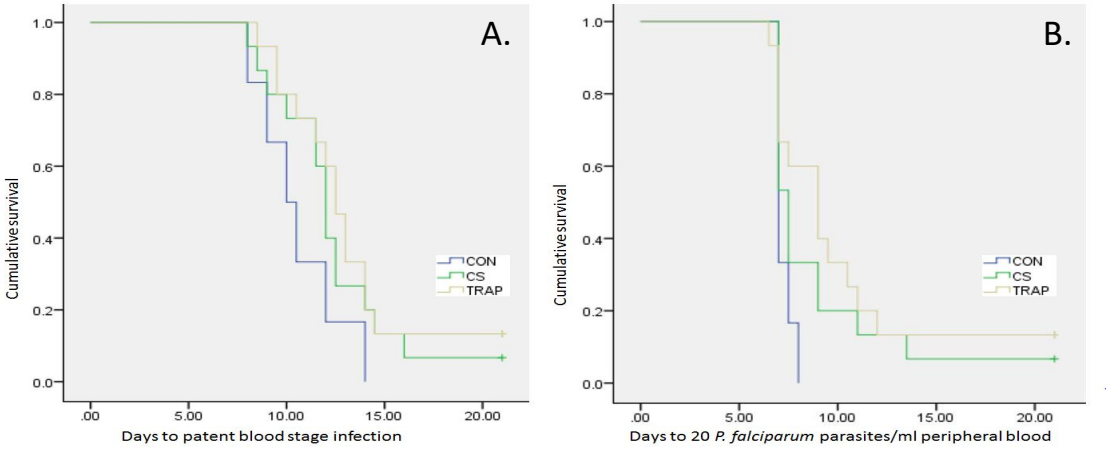


Figure 3: VAC045 clinical trial: Kaplan-Meier comparison of days to protocol-defined patent blood stage infection (A) or blood parasite level of 20 parasites/ml by quantitative PCR (B) following CHMI of VAC045 volunteers. Volunteers received ChAd63-MVA ME-TRAP vaccination (brown line; ChAd63 ME-TRAP 5 x 10^{10} vp IM followed by MVA ME-TRAP 2 x 10^{8} pfu eight weeks later), ChAd63-MVA CS vaccination (green line; ChAd63 CS 5 x 10^{10} vp IM followed by MVA CS 2 x 10^{8} pfu eight weeks later) or were unvaccinated controls (blue line). (A) Of 15 ChAd63-MVA ME-TRAP volunteers, 2 volunteers showed sterile protection (failure to develop blood stage infection by Day 21) and five further volunteers showed a delay in time to patent blood stage infection (to at least Day 13, 2.5 days beyond the mean for unvaccinated controls). Of 15 ChAd63-MVA CS volunteers, 1 volunteer showed sterile protection and three further volunteers showed a delay in time to patent blood stage infection. Patent blood stage infection was defined using a protocol-defined composite of clinical features, blood film result and quantitative *P. falciparum* PCR on peripheral blood. In (B), time of blood stage infection is determined using a threshold of 20 parasites/ml by quantitative *P. falciparum* PCR. Of 15 ChAd63-MVA ME-TRAP volunteers, 2 volunteers showed sterile protection and 7 further volunteers showed a delay in time to 20 parasites /ml (to at least Day 9, 1.75 days beyond the mean for unvaccinated controls). Of 15 ChAd63-MVA CS volunteers, 1 volunteer showed sterile protection and four further volunteers showed a delay in time to 20 parasites/ml.

The targeted mechanism of action is the induction of TRAP-specific $CD8^{+}$ T cells that eliminate *P. falciparum* infected hepatocytes. Low to moderate antibodies to TRAP are also induced but these have not yet been shown to correlate with vaccine efficacy. In the MAL034 study, vaccine efficacy was found to correlate with TRAP-

specific interferon-gamma-producing $CD8^+$ T cells (Figure 4A) and a threshold value of 0.2% $CD8^+$ TRAP-specific T cells was identified as a correlate of efficacy (Figure 4B). A preclinical model of the efficacy of ChAd63-MVA ME-TRAP prime-boost immunisation showed that vaccine-induced ME-TRAP-specific effector T cells could prevent the development of blood stage infection following *P. berghei* sporozoite challenge.[81] As T cell immunogenicity has progressively increased with changes in the vector platforms used for delivery of ME-TRAP, so too has vaccine efficacy (Table 1). The ChAd63-MVA heterologous prime-boost immunisation platform has induced the most potent $CD8^+$ T cell responses to date for any vaccination approach in clinical trials for malaria and has also been used to generate potent T cell responses in candidate vaccine immunisation strategies for Hepatitis C and HIV. Previously, concerns have been raised that vectored malaria vaccines may elicit lower T cell responses in malaria exposed populations compared with malaria naïve subjects,[82] however, more recent studies have shown that when administered IM and at optimal dose in a heterologous prime-boost sequence, ChAd63/MVA ME-TRAP can induce potent specific T cell responses in Kenyan and Gambian adults.[83] Furthermore, this regimen has been shown to induce partial protection against malaria parasitaemia by PCR in a study of 121 Kenyan male adults. In this study, the reduction in risk of malaria infection, as assessed by PCR monitoring over an 8 week period was 67% (95% CI 33-83%; P = 0.002).[84]

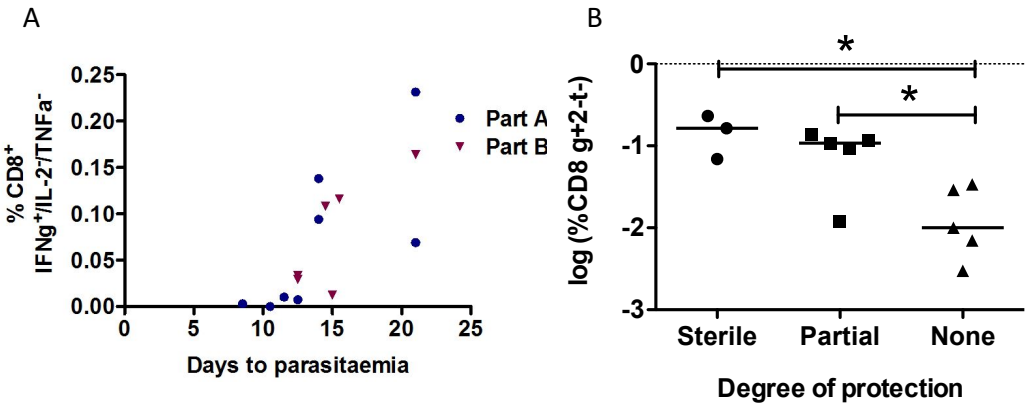


Figure 4: Study of the associations between immune responses and efficacy of ChAd63-MVA ME-TRAP vaccination in the MAL034 clinical trial.[79] Both cellular and antibody responses at the time of challenge were studied for their association with delay to patent parasitaemia. A: Correlation of time to parasitaemia with frequency of vaccine-induced $CD8^+$ T cells secreting IFNγ but not IL-2 or TNFα: $r_s = 0.81$, $p = 0.0005$. Volunteers underwent sporozoite challenge in one of two experiments (Part A and Part B). B: A secondary analysis of subgroups showed that frequencies of $CD8^+$ $IFN\gamma^+IL2^-TNF\alpha^-$ T cells before challenge were also significantly higher in vaccinees showing either partial or sterile protection than in non-protected vaccinees, P=0.009 across groups using 1 way ANOVA. * P<0.05 after Bonferroni multiple comparison test for comparison between groups.

Vaccine encoding ME-TRAP	T cell response mean cells/million PBMCS*	Efficacy
DNA x 3[77]	48	Nil
Fowl-pox x 2	50	Nil
MVA x 3[77]	41	Nil
DNA & MVA[77,85]	430	23%
Fowl-pox & MVA[86]	475	25%
ChAd63-MVA[79]	2400	58%

Table 1: Clinical trials of ME-TRAP encoding vaccines by University of Oxford, summarizing maximum T cell response as measured by IFNγ producing ELISPOT at peak time point post final boost, and clinical efficacy defined as sterile protection or significant delay to parasitemia post CHMI by mosquito bite.

1.4.13. Concept of a Multi-Stage/Multi-component Malaria Vaccine

A proposed method for increasing vaccine efficacy (VE) is combining antigenically distinct vaccines, targeting different stages of the parasite life-cycle, into a single regimen, and there is strong belief in the need for a multi-component malaria vaccine.[87-90] Different vaccine platforms exert efficacy against malaria through differing primary immune mechanisms,[24,25,79,91] and an additional benefit of combining vaccine types is that of exploiting both humoral and cellular immune responses to potentially increase efficacy.

1.4.13.7. RTS,S/AS in Combination Regimens With Either DNA Vaccines or Viral Vectored Vaccines

Optimal protection against malaria may require induction of high levels of protective antibody and $CD4^+$ and $CD8^+$ T-cell responses. It is reasonable to surmise, therefore, that an optimised vaccine approach may employ combination vaccine strategies. Malaria DNA vaccines, and more recently, vaccines delivered by viral vectors in heterologous prime-boost strategies elicit both $CD8^+$ cytotoxic T-cells and interferon gamma (IFNγ) responses, while recombinant proteins in adjuvant, such as RTS,S/AS, elicit high levels of NANP-specific antibodies and T-cell responses, but no CS-specific cytotoxic $CD8^+$ T-cells.[25,92,93] RTS,S has been evaluated in combination regimens with either DNA vaccination or viral vectored vaccines in two studies to date. One study,

published by Epstein et al in 2004 enrolled 27 volunteers, 10 of whom had previously received DNA vaccination via gene gun with a *P. falciparum* circumsporozoite protein (PfCSP) DNA vaccine in a study in 1999-2000.[33] These 10 volunteers, and an additional 14 healthy volunteers were all given a course of RTS,S/AS02$_A$ consisting of two doses of RTS,S/AS02$_A$ (50 μg) at 0 and 8 weeks. The vaccine was safe and well tolerated with no SAEs reported. Volunteers who had previously received PfCSP DNA vaccination did not have enhanced CS antibody responses following the course of RTS,S/AS02$_A$. This was is in contrast, however, to the CS-specific T cell responses in the same group, which were considerably enhanced in the DNA primed group.

The other study, conducted in Oxford in 2002, evaluated a heterologous prime-boost regimen of RTS,S/AS02$_A$ and MVA encoding CS in 18 healthy adult volunteers.[94] After enrolment, volunteers were randomised to one of two arms: "RRM" or "MRR". The two groups ran concurrently with RRM volunteers receiving two doses of RTS,S/AS02$_A$ (50 μg) followed by one dose of MVA CS at a dose of 1 x 10^8, and MRR volunteers receiving one dose of MVA CS followed by 2 doses of RTS,S/AS02$_A$ on a 0, 1 and 3 month schedule. Both groups, and a third group of unvaccinated infectivity control volunteers underwent sporozoite challenge via mosquito bite. Vaccines were well tolerated, with no SAEs. Anti-CS antibodies were present in both vaccine groups. Twelve vaccinated volunteers underwent sporozoite challenge, 6 from each vaccine group. Four of these volunteers (2 from each vaccine group) were completely protected against *P. falciparum* infection. For subjects diagnosed with malaria, there was a statistically significant delay to parasitaemia compared to

unvaccinated controls in the combined vaccine group, and in the MRR group, but not in the RRM group. All 4 volunteers displaying sterile protection underwent re-challenge five months later. Of these, one RRM subject was completely protected, with evidence of a delay to parasitaemia in one MMR subject. In this study, the detected effector T cell responses to CS on the day of challenge were low. It is unknown at this stage, however, whether enhanced T cell responses would be seen when both components of a viral vector prime–boost regimen, such as ChAd63-MVA ME-TRAP are co-administered with a full course of RTS,S/AS vaccine.

1.5. Microbial Challenge Studies of Human Subjects

Human challenge studies are studies in which human subjects are deliberately infected with micro-organisms and they are important tools in furthering our understanding of numerous disease mechanisms and their potential interventions. In contrast to animal challenge models, in which harm is an undesirable, but sometimes unavoidable outcome, human challenge studies are limited to diseases for which the risk to the participants is minimal. This is either due to the low virulence of the challenge infection, or the availability of reliable, effective treatment. Despite these limitations, human challenge models have been developed to evaluate a remarkable and diverse range of infectious diseases, including viral, bacterial and parasitic infections. Challenge studies provide proof of concept for prophylactic and therapeutic interventions with potential for significantly accelerating progress to larger interventional studies.[95]

1.5.1. Controlled Human Malaria Infection (CHMI)

P. falciparum malaria causes a disease that is particularly well suited to controlled infection studies, due to the highly predictable clinical course of disease, and the prompt response to effective, non-toxic treatment. Following inoculation with *P. falciparum*, subjects are asymptomatic during the relatively short pre-erythrocytic stage of disease. During the symptomatic period, thick film microscopy, in combination with clinical assessment and polymerase chain reaction (PCR) can be used to reliably diagnose clinical malaria and allow for the prompt initiation of curative therapy. In addition, no long-term sequelae or chronic infectious state occurs following completion of promptly administered treatment. Early examples of CHMI studies, performed in 1917 by Wagner von Jauregg, assessed the potential of symptomatic malaria infection as a treatment for patients with neurosyphilis.[96] More recently, however, studies have employed CHMI as a powerful tool for assessing malaria vaccine and prophylactic drug efficacy.[97] As the field of malaria vaccine research expands, there is a consequential increase in the number of centres conducting CHMI.[97]

Successful conduct of CHMI trials is dependent on a reliable source of live parasites, but historically, laboratory culture of *P. falciparum* has been problematic. In 1976 protocols were developed that documented techniques for the continuous culture of *P. falciparum*,[98] and subsequently for the generation of mature *P. falciparum* gametocytes *in-vitro* in 1981.[99] After successful demonstration of the infectivity of laboratory cultured gametocytes,[100] the first CHMI study to use laboratory-reared infectious mosquitoes was carried out by the US Walter Reed Army Institute of

Research (WRAIR), the US Naval Medical Research Institute (NMRI) and the US National Institutes of Health (NIH) in 1986.[101] This was shortly followed by publication of the efficacy of the first recombinant protein and synthetic peptide *P. falciparum* vaccines against CHMI using cultured parasites and laboratory reared mosquitoes.[102,103]

CHMI using laboratory reared mosquitoes is now firmly established as a key tool in assessing efficacy of novel malaria vaccines and drugs with many volunteers experimentally infected with between 1986 and the present.[104,97]

1.5.2. CHMI Delivered by Mosquito Bite

In order to generate an inoculation method that closely mimics natural infection, mosquito bite is traditionally used to deliver sporozoite-stage malaria parasites.[97] This is a favoured method to test pre-erythrocytic stage vaccines, using *Anopheles stephensi* mosquitoes that have fed on blood infected with *P.falciparum*. Commonly used strains of *P. falciparum* include the chloroquine-sensitive NF54 strain, the 3D7 clone of NF54, or less frequently, the 7G8 strain.[97,104] Nearly all unvaccinated volunteers bitten by five infected mosquitoes develop patent parasitaemia, with very rare exceptions. Infection rates drop significantly when volunteers are exposed to fewer than five infected mosquitoes. A major strength of the sporozoite infection model is the use of infectious mosquitoes, mimicking the natural route of infection.[97]

1.5.3. Conduct of CHMI Trials

In 2011 a document titled "Standardization of Design and Conduct of *P. falciparum* Sporozoite Challenge Trials" was published.[104] This was the result of an international collaborative consensus process between several academic and industry institutions that included the University of Oxford. Although some flexibility remains with regard to follow-up procedures in CHMI trials, there is consensus between groups on the following key points:

- All volunteers should have a medical assessment no longer than 48 hours before challenge, including an interim medical history, directed physical examination, and pregnancy test for female volunteers.
- Follow-up visits should be scheduled at least once daily, but may increase in frequency to two or three times daily, starting at day 5-7 post-challenge. At all visits volunteers should be questioned about the occurrence of adverse events and use of medication.
- In the event that a volunteer does not attend a scheduled follow-up visit it is imperative that investigators find that volunteer as quickly as possible and assess them for patent parasitaemia and clinical malaria. Should the volunteer withdraw consent from further follow-up prior to receipt of antimalarial drugs, it may be appropriate to withdraw the volunteer from the trial protocol and administer a course of antimalarial chemotherapy under close supervision.
- Grading and reporting of adverse events should be performed using international and local guidelines. It should be noted that the occurrence of a

low frequency of grade 3 severe adverse events, of short duration, and with no long-term sequelae, is not unexpected in CHMI studies. A minority of those challenged are known to experience grade 3 systemic adverse events and this fact should be included in the informed consent form.

- Vital signs should be recorded at least once daily and at any subsequent visits for medical attention. Directed physical examination should be performed when necessary.
- It is critical that every volunteer must receive every dose of anti-malarial therapy. In some settings fully directly observed treatment will be essential. Where directly observed treatment is not used, investigators must follow volunteers closely to ensure compliance with the treatment regimen.
- After challenge, all volunteers should be followed until they have completely finished anti-malaria treatment.
- Volunteers should be evaluated at least two weeks after finishing treatment.
- A local safety monitor and an independent safety monitoring committee should be established to act as independent experts in evaluating adverse events. The safety monitor or monitoring committee may advise the investigators on initiating anti-malarial treatment for a specific volunteer or volunteer group. While safety monitoring committees are not a requirement for Phase 1 trials, they should be considered a requirement for CHMI trials which have an efficacy component and which have major potential safety concerns.

1.5.4. Ethical Considerations of CHMI Trials

Participants are invited to volunteer for CHMI trials in the understanding that they will not obtain direct health benefit from participation. A detailed assessment of each subject's current and previous medical status must be made prior to participation to ensure that they are healthy volunteers. CHMI trial investigators are obliged to exercise all possible safeguards to ensure minimal risk and maximise volunteer safety. Investigators must also ensure that maximal scientific benefit accrues from each challenge trial. Key consensus ethical considerations include:[104]

- Volunteer safety is the paramount consideration in conduct of CHMI trials.
- Adherence to both international and local guidelines with respect to ethical considerations and in accordance with the Declaration of Helsinki and local regulatory and ethics committee requirements.
- CHMI trials should be conducted according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and/or WHO Good Clinical Practice guidelines. with the aim of maximising scientific benefit whilst minimising risk.
- The raw data (both microscopy and PCR where available) from challenge trial datasets should be made publicly available to facilitate scientific benefit to the community.
- If an unexpected serious adverse event (SAE) which is possibly related to CHMI occurs at a challenge trial centre, recognising legal restrictions, every effort should be made to communicate information on this SAE to the

community of challenge trial centres within 90 days of the occurrence of the SAE.

1.5.5. Clinical Presentation Following CHMI

Severe or life-threatening malaria has never occurred following CHMI.[105] All subjects in CHMI trials are counselled to that they may develop symptoms of clinical malaria infection and that approximately one-fifth of volunteer's symptoms that would temporarily be graded as severe (ie. symptoms that prevent daily activities).

Follow up laboratory checks generally show moderate leukopenia and thrombocytopenia during malaria infection, with no change in haemoglobin concentration.[106] Bleeding or thrombogenic complications have never been described.[105,106] Transient abnormalities of liver enzymes have been observed, but these abnormalities did not result in clinical manifestations and resolved spontaneously.

The risks of severe malaria complications must be minimised by immediate curative treatment at the earliest phase of microscopically detectable parasitaemia. Recently, safety concerns were raised in a young volunteer who suffered a cardiac event shortly after treatment for diagnosed malaria. This was diagnosed as probable myopericarditis, although ischaemia could not be ruled out. Although a definite relationship between the cardiac event, which resolved fully and rapidly, and the experimental malaria infection was not established,[107] it has been generally agreed that volunteers with an increased risk of cardiac disease should be excluded from such trials.[104] A further case of myopericarditis has since been identified in a recent

CHMI study, also at the Nijmegen, Netherlands centre, but in this case the individual was also diagnosed with an intercurrent rhinovirus infection so that the relation to malaria infection is again uncertain. There was a brief episode of clinical chest pain and the volunteer made a full recovery.[108]

1.6. Ebola

1.6.1. History of Ebola Virus Discovery

Ebolavirus disease (EVD) is caused by the Ebolavirus and it was first reported in 1976 in simultaneous outbreaks in Sudan and Democratic Republic of Congo (DRC). It is named after the Ebola river in the DRC, near the village where the outbreak occurred.[109]

1.6.2. Virology and Pathogenesis

The Filoviridae family contains 3 genera that are known to induce VHF: Ebolavirus, Marburgvirus and Cuevavirus. Within the Ebolavirus genus, there are 5 species: Bundibugyo (BDBV), Reston (RESTV), Sudan (SUDV), Taï Forest (TAFV), and Zaire (EBOV) [110], of which BDBV, EBOV, and SUDV have caused large outbreaks with high case fatality rates in Africa.[109] EVD in humans was formerly known as Ebola haemorrhagic fever (EHF).

Ebolavirus is a large, negative-strand RNA filovirus. It has a genome composed of 7 genes encoding 7 structural proteins, and 1 non-structural protein. These include a glycoprotein (GP) that has a secretory and transmembrane form.[111-113] The animal reservoir for Ebolavirus has never been confirmed, but fruit bats are suspected.[114] The transmission of Ebolavirus from animal reservoir to humans is not yet fully understood, but is likely due to incidental exposure to infected animal tissues and fluids.[115-117] Outbreaks occur through person-to-person transmission, with infection resulting from direct contact with blood, or other bodily fluids of infected people, or

indirect contact with contaminated environments.[109] Ebolavirus has been isolated from semen as long 2 years after acute infection.[109,118] It is unclear, however, whether patients remain infectious for as long as their blood and secretions contain the virus. The high infectivity of blood and secretions puts healthcare workers at particularly high risk during outbreaks, and direct contact with the bodies of deceased victims can also have a role in the transmission of the virus.

The incubation period of EVD is 2 to 21 days (approximately 7 days on average, but strain dependent) followed by an acute viral illness often characterised by the rapid onset of non-specific symptoms such as fever, fatigue, myalgia, arthralgia headache and sore throat. This is followed by vomiting, diarrhoea, rash and symptoms of impaired kidney and liver function. In some cases severe symptoms related to haemorrhagic fever may occur after 2 to 7 days, including haemorrhagic rash, epistaxis, mucosal bleeding, haematuria, haemoptysis, gastrointestinal bleeding, conjunctival haemorrhage, tachypnoea, confusion, somnolence, and hearing loss.[109] Haemorrhagic symptoms were less frequent during the 2014 West African outbreak, where severe gastrointestinal symptoms were commonly reported.[119] Laboratory findings include leucopenia, thrombocytopenia and elevated liver enzymes.[109] Symptoms last for approximately 7 to 14 days after which recovery or death may occur. Mortality rate in the 2014 West African outbreak ranged from 28% (Sierra Leone) to 67% (Guinea). Mortality in some outbreaks has been as high as 90%.[109,115,120]

Antibody responses can be detected in the blood from day 2 after infection. Appearance of IgG generally coincides with the recovery phase, from day 17 onwards, but the contribution of either humoral and cellular immunity to protection against EVD remains unknown at present.[121]

1.6.3. The 2014 West African Ebola Outbreak

The 2014 Ebola virus disease (EVD) outbreak was first recognised in Guinea in March 2014, and as of December 2014, cases and deaths had been reported in six West African countries. Guinea, Liberia and Sierra Leone were the worst affected, with cases also reported in Nigeria, Senegal and Mali. Furthermore, onward transmission from imported cases occurred in Spain and the USA. The United Kingdom reported its first newly diagnosed case of EVD on the 29th December 2014. The known case rate far exceeded the largest prior EVD outbreak in Uganda in 2000 - 2001[122], with over 28 000 confirmed, probable or suspected cases, and over 11 000 deaths.[1]

1.6.4. The need for an Ebola vaccine

EVD has occurred in numerous sporadic outbreaks since it was discovered in 1976. Although the total number of cases to date remain relatively few, the extremely high mortality and ease of transmission, result in a high level of global concern around the disease. Furthermore, during severe outbreaks, such as that in West Africa, those at greatest risk are those in close contact with infected victims. This includes healthcare, burial and government and non-government aid workers. Viruses in the Filoviridae family are also categorized as potential threats for use as biological

weapons due to ease of dissemination and transmission, and high levels of mortality. Currently, no effective therapies or FDA-licensed vaccines exist for any member of Filoviridae family of viruses. Development of an effective vaccine against these viruses would maximise safety of those at greatest risk of disease during outbreaks.

1.6.5. Development of Ebola Vaccines

The first attempts at vaccine development for EBOV utilized inactivated virus. Although efficacy in guinea pigs infected with ZEBOV had been demonstrated, this efficacy did not translate to a nonhuman primate (NHP) challenge model.[123,124] Classical subunit vaccines such as recombinant expressed purified viral proteins have only been shown to be partially protective in rodent models for EBOV infection, and further improvement of immunogenicity and efficacy would be needed to justify efficacy studies in the expensive and ethically complex macaque challenge model.[125]

Further approaches have evaluated the immunogenicity and efficacy of alphavirus and flavivirus replicons,[126,127] DNA vaccines,[128-133] and both replication competent and replication deficient viral vectors including adenoviruses,[51,134-140] and vesicular stomatitis virus (VSV) vectors.[141-147]

1.6.6. Previous Experience with VRC Filovirus Vaccines

Prior to the development of ChAd3-EBO Z the US National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Centre (VRC) had developed 3 different investigational Ebolavirus vaccines and a single Marburg virus vaccine. These have been evaluated in a series of Phase 1 clinical trials. The vaccines have been shown to

be immunogenic in humans, and no safety concerns have been raised. Clinical trials of these vaccines are summarised in Table 2.[148]

Study Identifier (Clinicaltrials.gov)	**Study Design**	**Vaccine Product(s)**	**Dosage, route, x N administrations**	**Accrual* Product/ Placebo**
VRC 204 [130] (NCT00072605)	Phase I, randomised, placebo-controlled, dose escalation	VRC-EBODNA012-00-VP (Ebola DNA, ΔTM GP)	2 mg IM x 3 doses 4 mg IM x 3 doses 8 mg IM x 3 doses	**5**/2 **8**/2 **8**/2
VRC 205 [135] (NCT00374309)	Phase I, randomised, placebo-controlled, dose escalation	VRC-EBOADV018-00-VP (Ebola-rAd5, PM GP)	$2x10^9$ vp† IM x 1 dose $2x10^{10}$ vp IM x 1 dose	**12**/4 **12**/4
VRC 206 [149] (NCT00605514)	Phase I, open label	VRC-EBODNA023-00-VP (Ebola DNA, WT GP) VRC-MARDNA025-00-VP (Marburg DNA, WT GP)	4 mg IM x 3 or 4 doses 4 mg IM x 3 or 4 doses	**10/**0 **10**/0
RV 247 (NCT00997607)	Phase Ib, randomised, placebo-controlled	VRC-EBODNA023-00-VP VRC-MARDNA025-00-VP VRC-EBODNA023-00-VP and VRC-MARDNA025-00-VP	4 mg IM x 3 doses 4 mg IM x 3 doses 4 mg IM x 3 doses; both vaccines	**30**/6 **30**/6 **30**/6
* number of subjects received Ebola vaccine is shown in bold † viral particles				

Table 2: Summary of the VRC Studies for Evaluation of Ebola Vaccines. Table adapted from NIH clinical trial protocol VRC 207[148]

The first investigational vaccine was a 3-plasmid recombinant DNA vaccine encoding for nucleoprotein (NP) from the Zaire strain of Ebola and for transmembrane deleted (ΔTM) Ebola GP sequences from Zaire and Sudan strains (VRC-EBODNA012-00-VP). The inclusion of the ΔTM GP sequence was designed to address theoretical concerns regarding cellular toxicity observed during *in vitro* experiments when plasmids

expressing full length, wild-type (WT) GP proteins were transfected into cell culture.[148,150]

Following this, a replication-defective, recombinant adenovirus serotype 5 vaccine encoding GP genes (Ebola-rAd5) with an E71D point-mutation (aspartic acid for glutamic acid at position 71) was assessed. This had been demonstrated as being safe and immunogenic in animal studies without toxicity. A rAd5 vaccine encoding for E71D EBOV GP combined with E71D SUDV GP had demonstrated efficacy in macaques against challenge EBOV. Ebola-rAd5 vaccine was shown to be immunogenic in humans, but pre-existing anti-vector immunity to Ad5 was thought to impact antibody responses against Ebolavirus GP.[135,148]

Concurrent studies in non-human primates (NHPs) showed that WT GP constructs provided higher levels of protection than ΔTM or E71D constructs.[151] Therefore, the WT GP antigen subsequently became the focus of Ebola vaccine research and development at VRC/NIAID. Subsequent assessment of WT GP constructs has not been associated with any safety concerns. Evaluation of simian adenovirus and MVA vectors encoding EBOV WT GP was considered to be a promising approach.[148]

1.6.7. ChAd3-EBO Z

As of 2014 the NIAID/VRC, in collaboration with the Italian biotech company, Okairos, had developed a bivalent recombinant chimpanzee adenovirus Type 3-vectored Ebolavirus vaccine cAd3-EBO (also denoted ChAd3-EBO). The vaccine consists of a mixture of 2 ChAd3 vectors encoding for wild type (WT) Ebola virus

glycoprotein (GP) from the Zaire [GP (Z)] and Sudan [GP (S)] species of Ebola virus. The monovalent form targeting the outbreak Zaire strain is denoted ChAd3-EBO Z.

The efficacy of the ChAd3 vector encoding both the EBOV and SUDV GPs, and in either monovalent or bivalent preparations, was evaluated in cynomologous macaques against lethal EBOV challenge. Encouragingly, all vaccinated macaques were protected against the EBOV challenge with no detectable viraemia, whilst all unvaccinated controls succumbed to infection.[152] Furthermore, durable protection was observed to EBOV challenge 10 months after initial vaccination when a heterologous boosting vaccination of MVA expressing both GPs was administered 8 weeks after ChAd3. This effect was not seen when a boosting vaccination with either the same vector (ChAd3) or a different chimpanzee adenovirus serotype (ChAd63) was given at 8 weeks following ChAd3 prime vaccination.

1.6.8. Previous Experience with Chimpanzee Adenovirus Vector Serotype 3 (ChAd3)

A recombinant chimpanzee adenovirus serotype 3 (ChAd3) vector HCV vaccine has been evaluated in human subjects, and was shown to be safe and well tolerated. The vaccinations induced HCV-specific immune responses that were sustained for at least a year after the boost with a heterologous adenoviral vector.[153]

ChAd3 is a subgroup C adenovirus with low seroprevalence in human sera.[154] (unpublished VRC data). ChAd3 based vaccines induce comparable immunogenicity to rAd5 vectors,[155,156] and pre-existing anti-rAd5 immunity does not appear to cross-

react with ChAd3 in mice.[155] [unpublished VRC data]. After consideration of both ChAd3 and ChAd63 vectors, the ChAd3 vector was chosen for development into an investigational EBOV vaccine for human clinical trials.

1.6.9. MVA-BN® Filo

The multivalent vectored vaccine MVA-BN® Filo contains an insert for the Zaire Ebola virus glycoprotein (EBOV GP), as well as the Sudan strain Ebola virus glycoprotein (SUDV GP), the Musoke strain Marburg virus glycoprotein, and a nucleoprotein from the Tai-Forest Ebola virus (TAFV NP). Non-human, pre-clinical studies have raised no safety concerns, and it was shown to be immunogenic across all species tested. Furthermore, a heterologous prime-boost regimen of MVA-BN® Filo in combination with a triple vector Ad26 mix (a mixture of Ad26 encoding EBOV GP, Ad26 encoding SUDV GP and Ad26 encoding Marburg glycoprotein) in non-human primates resulted in complete protection against lethal challenge with Zaire Ebola, independently of the order in which they were administered (unpublished data from manufacturer's investigator brochure for MVA-BN® Filo). Nonclinical repeated dose toxicity studies and two biodistribution studies were conducted using the intramuscular and subcutaneous route of administration for MVA-BN®. These studies showed similar results between the intramuscular and subcutaneous dose administration groups. In terms of vaccine efficacy in the non-clinical setting, several recombinant MVA-BN® products have been tested via the subcutaneous and intramuscular route previously in mice and also non-human-primates with comparable or even slightly increased immunogenicity afforded by intramuscular vaccination.

Bavarian Nordic performed two Phase I clinical trials (POX-MVA-001 and POX-MVA-002), administering the MVA-BN® vector via subcutaneous and intramuscular routes in different study arms (POX-MVA-001: 18 out of total 86 subjects had intramuscular injections; POX-MVA-002: 15 out of total 90 subjects had intramuscular injections). In both trials, the parameters of safety and immunogenicity/efficacy were comparable between the intramuscular and subcutaneous study arms.[157,158]

1.6.10. Rationale

A vaccination strategy to achieve protective immunity in most recipients with a single vaccination would be desirable in an outbreak setting. Vaccination strategies that achieve durable protective immunity would be desirable for populations in areas of the world where outbreaks occur sporadically. Optimally, one approach would serve both needs, but a different approach may be needed for rapid immunity than is needed for durable immunity.

1.7. Aim of Work

At the time of starting the work detailed in this thesis, no licensed vaccine existed for either malaria or Ebolavirus disease. Vaccines for malaria had only demonstrated limited efficacy against CHMI, and few attempts had been made to improve efficacy by exploiting multiple facets of the immune system and targeting multiple stages of the parasite life cycle. Therefore, this work was conducted to evaluate novel malaria vaccine regimens and dosing schedules in malaria naïve adults that incorporated two distinct vaccine types and assess their safety, efficacy and immunogenicity in a CHMI model. Furthermore, for a malaria vaccine to be realistically deployable, a schedule that limits the overall number of vaccine doses would be desirable. Therefore this work evaluates concomitant administration of RTS,S/AS01B with viral vectors encoding ME-TRAP in an attempt to rationalise total numbers of vaccine administration visits within a schedule. In addition, an early study of RTS,S found that a group that received a fractional third dose ($1/5^{th}$ standard dose) in a 0, 1, 7 month schedule, exhibited higher efficacy against CHMI. Therefore, this work also sought to evaluate the impact of a fractional third dose of RTS,S/AS01B on efficacy when delivered in a 0, 1, 2, month schedule.

Several viral vector Ebola virus vaccines have shown promise in pre-clinical studies and the non-human primate challenge model. In the context of an evolving outbreak of Ebola virus disease in West-Africa, this work also sought to evaluate the safety and immunogenicity of the novel Ebola virus vaccine ChAd3-EBO Z either alone, or in a heterologous prime-boost regimen with the multivalent MVA-BN® Filo. For a vaccine targeting outbreak pathogens, rapid induction of protective immunity is

desirable. The impact of shortening the prime-boost interval in order to accelerate the induction of specific immune responses was also evaluated.

To summarise, the aims of this thesis were therefore:

- To establish whether the efficacy of a 3 dose schedule of RTS,S/AS01B in healthy malaria naïve adults against CHMI could be improved by the addition of ChAd63 ME-TRAP and MVA ME-TRAP in staggered administration in the same schedule.
- To establish whether this efficacy was durable at 6 months after initial CHMI by re-challenging protected vaccinated subjects with repeat CHMI.
- To establish whether a vaccine schedule incorporating 3 doses of RTS,S/AS01B and ChAd63 ME-TRAP and MVA ME-TRAP could be rationalized by concomitant administration of RTS,S/AS01B and viral vectors without compromising safety and efficacy.
- To evaluate the impact on safety, efficacy and immunogenicity of a reduced 3rd dose of RTS,S/AS01B in a 0, 1, 2-month schedule either with or without concomitantly administered viral vectors expressing ME-TRAP
- To examine the safety and immunogenicity of a novel viral vector Ebola vaccine (ChAd3-EBO Z) in a dose ranging first-in-man study.
- To evaluate whether the immunogenicity of a single dose of ChAd3-EBO Z can be improved by the addition of a heterologous boost with the multivalent filoviral vaccine MVA-BN® Filo, without compromising safety.
- To evaluate the impact on safety and immunogenicity of reduced prime boost intervals, as short as 1 week.

1.8. Overview of Thesis Chapters

Chapter 2: Describes materials and methods.

Chapter 3: Describes the safety, efficacy and immunogenicity of a staggered combination malaria vaccine schedule of RTS,S/AS01B with ChAd63 & MVA vectors expressing ME-TRAP.

Chapter 4: Describes the safety, efficacy and immunogenicity of a malaria vaccine schedule of RTS,S/AS01B concomitantly delivered with ChAd63 & MVA vectors expressing ME-TRAP, and also the effect of a fractional third dose of RTS,S/AS01B.

Chapter 5: Describes the safety and immunogenicity of the novel Ebolavirus vaccines ChAd3-EBO Z and MVA BN-Filo

Chapter 6: Presents conclusions and closing remarks

Appendices

References

Chapter 2

Materials and Methods

2. Chapter 2: Materials and Methods

2.1. Investigational Medicinal Products (IMPs)

2.1.1. Manufacturing and Presentation of Vaccines

2.1.1.8. RTS,S/AS01B

The candidate RTS,S/AS01B vaccine has been developed and manufactured by GSK Vaccines.

The vaccines were labelled and packed according to applicable regulatory requirements.

Treatment name	Vaccine/ product name	Formulation	Presentation	*Injectable volume*	Number of doses
RTS,S/AS01B	RTS,S	RTS,S antigen: 50 µg RTS,S with sucrose as cryoprotectant	RTS,S antigen: Lyophilized pellet in single dose vial to be reconstituted with AS01B adjuvant in liquid form	0.5 ml[%]	3
	AS01B	AS01B adjuvant: 50 microgrammes of MPL and 50 microgrammes of Stimulon QS21 (a triterpene glycoside purified from the bark of Quillaja saponaria) with liposomes	AS01B adjuvant: liquid solution in monodose glass vial		

[%] Volume after reconstitution

Table 3: Formulation and presentation of RTS,S/AS01B. Table provided by GSK vaccines.

2.1.1.9. Supply of RTS,S/AS01B

RTS,S/AS01B was supplied to the Clinical Biomanufacturing Facility (CBF), University of Oxford by GSK Vaccines, Belgium, in pre-prepared vials for reconstitution prior to vaccination. It was labelled for investigational use only by the CBF, who then

transferred the vaccines to the clinical sites. Vials of RTS,S were certified for release for use in this trial by a qualified person (QP) at the CBF, University of Oxford.

2.1.1.10. Storage of RTS,S/AS01B

All study vaccines were stored in a safe and locked place with no access by unauthorised personnel. The RTS,S/AS01B vaccine was stored at the defined temperature range (i.e. +2 to +8°C/36°F to 46°F).

Any temperature deviations outside the range 0 to 8°C/32 to 46°F were reported to the sponsor as soon as detected.

In case of such temperature deviations between 0 and 2°C/32 to 36°F the impacted study vaccines could still be administered following discussion with the chief investigator, but the site was required to take adequate actions to go back to the defined range +2 to +8°C/36 to 46°F and avoid re-occurrence of such a temperature deviation.

2.1.1.11. ChAd63 ME-TRAP

The recombinant chimpanzee adenovirus Type-63 vectored *P. falciparum* malaria vaccine (ChAd63 ME-TRAP) consists of a recombinant replication-deficient adenovirus chimpanzee serotype 63 (ChAd63) vector expressing the liver stage antigen TRAP fused to a multiple epitope string (ME). ChAd63 ME-TRAP was manufactured under Good Manufacturing Practice (GMP) conditions by the CBF, University of Oxford. ChAd63 ME-TRAP was supplied as a liquid in sterile aliquots in 2.0 mL clear glass vials.

2.1.1.12. MVA ME-TRAP

The recombinant Modified Vaccinia (MVA) Ankara *P. falciparum* malaria vaccine (MVA ME-TRAP) consists of a recombinant replication-deficient MVA vector expressing ME-TRAP. MVA ME-TRAP was manufactured under Good Manufacturing Practice conditions by IDT Biologika GmbH (IDT), Germany. MVA ME-TRAP was supplied as a liquid formulation in 10mM Tris buffer 140 mM NaCl, pH 7.7. The virus suspension was supplied as sterile aliquots in 2.0 mL clear glass injection vials. Final QP certification and associated labelling took place at the CBF, University of Oxford.

2.1.1.13. ChAd3-EBO Z

The recombinant chimpanzee adenovirus Type-3 vectored Ebola Zaire vaccine (ChAd3-EBO Z) consists of a recombinant replication-deficient adenovirus chimpanzee serotype 3 (ChAd3) vector expressing wild-type (WT) Ebola glycoprotein (GP) from the Zaire strain of Ebolavirus (EBOV). The drug substance was manufactured under Good Manufacturing Practice (GMP) conditions by ADVENT S.r.l. (Rome, Italy, under contract to GlaxoSmithKline [GSK] and the NIH). ChAd3-EBO Z was supplied as a liquid in sterile aliquots in 3 mL clear glass vials at a concentration of 3.6×10^{10} vp per ml.

2.2. MVA-BN® Filo

The multivalent modified vaccinia virus Ankara vectored vaccine (MVA-BN® Filo) was developed by Bavarian Nordic and contract manufactured under GMP conditions by Impfstoffwerk Dessau-Torman (IDT), Germany. MVA-BN® Filo was supplied as a

liquid formulation in TRIS and Sodium Chloride. The virus suspension was supplied as sterile aliquots in 2 mL clear glass injection vials.

2.2.1.14. Supply of Viral Vectored Vaccines

ChAd63 ME-TRAP was supplied to the clinical sites by the CBF, University of Oxford, where the vaccines were manufactured, vialed and labelled for investigational use only.

MVA ME-TRAP was supplied to the CBF, University of Oxford, by IDT, Germany where the vaccine was manufactured and vialed. Final QP certification and associated labelling to trial was then performed by the CBF, who then transferred the vaccines to the clinical sites.

ChAd3-EBO Z was filled, and supplied to the CBF by the VRC Vaccine Pilot Plant (VPP), operated by the Vaccine Clinical Materials Program, Leidos Biomedical Research, Inc., Frederick, MD. Final batch certification and associated labelling took place at the CBF, University of Oxford.

MVA-BN® Filo was supplied to the CBF, University of Oxford, by IDT, Germany where the vaccine was manufactured and vialed. Final batch certification and associated labelling took place at the CBF, University of Oxford.

All vaccines were certified for release by a qualified person (QP) at the CBF, University of Oxford.

2.2.1.15. Storage of Viral Vectored Vaccines

ChAd63 ME-TRAP and MVA ME-TRAP vaccines were stored between –65°C and –90°C. All movements of the study vaccines were documented. Vaccine accountability, storage, shipment and handling was in accordance with local SOPs.

2.3. Treatment of Trial Volunteers

2.3.1. Recruitment & Withdrawal of Trial Volunteers

Volunteers were recruited for all 3 trials by use of an advertisement +/- registration form formally approved by the ethics committee(s) for each trial and distributed or posted in the following places:

- In public places, including buses and trains, with the agreement of the owner/proprietor
- In newspapers or other literature for circulation
- On radio via announcements
- On a website operated by the investigators' clinical trials group or with the agreement of the owner or operator (including on-line recruitment through our web-site)
- a post on a Twitter or Facebook or other similar account owned and operated by the investigators' clinical trials group
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation.
- By email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the Oxford Vaccine Centre (all trials), NIHR

WTCRF Southampton **(VAC055 & VAC059 only),** the Surrey Clinical Research Centre, Guildford **(VAC059 only),** or the Hammersmith NIHR/Wellcome Trust Imperial CRF, London **(VAC055 & VAC059 only).**

- On stalls or stands at exhibitions or fairs
- Via presentations (e.g. presentations at lectures or invited seminars)
- Direct mail-out: This involved obtaining names and addresses of adults via the most recent Electoral Roll. The contact details of individuals who had indicated that they do not wish to receive postal mail-shots were removed prior to the investigators being given this information. The company providing this service is registered under the Data Protection Act 1998. Investigators were not given dates of birth or ages of individuals but the list supplied only contained names of those meeting the age criteria set for each trial.
- Oxford Vaccine Centre databases: Individuals from databases of groups within the CCVTM, (including the Oxford Vaccine Centre database) of previous trial participants who have expressed an interest in receiving information about all future studies for which they may be eligible were contacted.
- Southampton WTCRF Database of Healthy Volunteers: Individuals from this database who have previously expressed an interest in receiving information about future studies for which they may be eligible were contacted. **(VAC055 & VAC059 only)**
- Hammersmith NIHR Wellcome Trust CRF Database of Healthy Volunteers: Individuals from this database who have previously expressed an interest in

receiving information about future studies for which they may be eligible were contacted. **(VAC055 & VAC059 only)**

- Surrey Clinical Research Centre Database of Healthy Volunteers: Individuals from this database who have previously expressed an interest in receiving information about future studies for which they may be eligible were contacted. **(VAC059 only)**

2.3.2. Informed Consent

All volunteers were required to sign and date the informed consent form before any study specific procedures were performed. The information sheet was made available to the volunteer at least 24 hours prior to the screening visit. At the screening visit, the volunteer was fully informed of all aspects of the trial, the potential risks and their obligations. The following points were emphasised:

- Participation in the study was entirely voluntary.
- Refusal to participate involved no penalty or loss of medical benefits.
- The volunteer may have withdrawn from the study at any time. However, if the volunteer had undergone CHMI **(VAC055 & VAC059 only)** and not completed a course of appropriate antimalarial therapy then the volunteer would have needed to maintain contact with the investigators for monitoring and treatment.
- The volunteer wss free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved.
- There was no direct benefit from participating.

- The study involved research of an investigational vaccine
- The volunteer's GP would be contacted to corroborate their medical history and confirm that the volunteer was eligible to take part in the study. Volunteers were only enrolled in the study if written or verbal information regarding the volunteer's medical history was obtained from the GP.
- The volunteer was registered on the TOPS database (The Overvolunteering Prevention System; www.tops.org.uk).

The aims of the study and all tests to be carried out were explained. The volunteers were given the opportunity to ask about details of the trial, and were given time to consider whether or not to participate. If they decided to participate, volunteers in the VAC055 & VAC059 trials were asked to complete a questionnaire testing their understanding of the trial, thereby ensuring that individuals understood the trial sufficiently to give informed consent. Volunteers were required to answer all questions in the questionnaire correctly before proceeding, but in the case of failing on their first attempt, volunteers could re-take the questionnaire only following further discussion with the investigator. Volunteers in all trials were then asked to sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the case report form (CRF). These forms were also signed and dated by the Investigator.

2.3.3. Inclusion and Exclusion Criteria

2.3.3.16. Inclusion Criteria

The volunteer was required to satisfy all the following criteria to be eligible for the studies:

- Healthy adults aged 18 to 45 years (**VAC055 & VAC059**) or 18 to 50 years (**EBL01**).
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner.
- For females only, willingness to practice continuous effective contraception (see section 2.3.4) during the study and a negative pregnancy test on the day(s) of screening and vaccination
- Agreement to refrain from blood donation during the course of the study and (**VAC055 & VAC059 only**) for at least 3 years after the end of their involvement in the study.
- Written informed consent to participate in the trial.
- Reachable (24/7) by mobile phone during the period between CHMI and completion of antimalarial treatment. (**VAC055 & VAC059 only**)
- Willingness to take a curative anti-malaria regimen following CHMI. (**VAC055 & VAC059 only**)
- For volunteers not living in Oxford: agreement to stay in a hotel room close to the trial centre during a part of the study (from at least day 6.5 post

mosquito bite until anti-malarial treatment is completed). (**VAC055 & VAC059 only**)

- Answer all questions on the informed consent quiz correctly. (**VAC055 & VAC059 only**)

2.3.3.17. Exclusion Criteria

The volunteer could not enter the study if any of the following applied:

- History of clinical malaria (any species). (**VAC055 & VAC059 only**)
- Travel to a malaria endemic region during the study period or within the preceding six months with significant risk of malaria exposure. (**VAC055 & VAC059 only**)
- Use of systemic antibiotics with known antimalarial activity within 30 days of CHMI (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin) (**VAC055 & VAC059 only**)
- Receipt of an investigational product in the 30 days preceding enrolment, or planned receipt during the study period.
- Prior receipt of an investigational malaria vaccine (**VAC055 & VAC059 only**) or Ebola or Marburg vaccine (**EBL01**) or any other investigational vaccine likely to impact on interpretation of the trial data. For volunteers in VAC055 Group 1 and 2 who underwent re-challenge, this exclusion criterion did not extend to the vaccines previously received in the VAC055 trial
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic

(more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids excepted).

- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine (e.g. egg products, Kathon) or malaria infection.
- Any history of anaphylaxis post vaccination.
- History of clinically significant contact dermatitis.
- History of sickle cell anaemia, sickle cell trait, thalassaemia or thalassaemia trait or any haematological condition that could affect susceptibility to malaria infection. (**VAC055 & VAC059 only**)
- Pregnancy, lactation or intention to become pregnant during the study.
- Use of medications known to cause prolongation of the QT interval ***and*** existing contraindication to the use of Malarone (**VAC055 & VAC059 only**)
- Use of medications known to have a potentially clinically significant interaction with Riamet ***and*** Malarone (**VAC055 & VAC059 only**)
- Any clinical condition known to prolong the QT interval (**VAC055 & VAC059 only**)
- History of cardiac arrhythmia, including clinically relevant bradycardia (**VAC055 & VAC059 only**)
- Disturbances of electrolyte balance, eg, hypokalaemia or hypomagnesaemia (**VAC055 & VAC059 only**)
- Family history of congenital QT prolongation or sudden death (**VAC055 & VAC059 only**)

- Contraindications to the use of all three proposed anti-malarial medications; Riamet, Malarone and Chloroquine. (**VAC055 & VAC059 only**)
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of serious psychiatric condition
- Any other serious chronic illness requiring hospital specialist supervision.
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 standard UK units every week.
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Hepatitis B surface antigen (HBsAg) detected in serum.
- Seropositive for hepatitis C virus (antibodies to HCV) at screening.
- An estimated, ten-year risk of fatal cardiovascular disease of ≥5%, as estimated by the Systematic Coronary Risk Evaluation (SCORE) system. (**VAC055 & VAC059 only**)
- Positive family history in 1st and 2nd degree relatives < 50 years old for cardiac disease. (**VAC055 & VAC059 only**)
- Volunteers unable to be closely followed for social, geographic or psychological reasons. (**VAC055 & VAC059 only**)
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination. In the event of abnormal test results, confirmatory repeat tests will be requested.
- Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect

the ability of the volunteer to participate in the study or impair interpretation of the study data.

- Inability of the study team to contact the volunteer's GP to confirm medical history and safety to participate
- Travel to an Ebola or Marburg endemic region during the study period or within the previous six months **(EBL01 only)**

2.3.4. Effective contraception for female volunteers

Female volunteers were required to use an effective form of contraception during the course of each study. As these vaccines had not yet been assessed in pregnancy there was no information about the effect of the vaccine on a foetus. Previous investigations looking at excretion of similar adenoviral viral vectors after vaccination in urine for males demonstrated no detectable virus, and therefore males were not required to use barrier contraception whilst taking part in this study as the risk of excretion of the virus is very low.

2.3.5. Prevention of "over-volunteering"

Volunteers were excluded from the study if they were concurrently involved in another trial. In order to check this, volunteers were asked to provide their National Insurance or Passport number (if they were not entitled to a NI number) and were registered on a national database of participants in clinical trials (*www.tops.org.uk*).

2.3.6. Compensation of volunteers

Volunteers were compensated on a pro-rata basis for their time and for the inconvenience caused by procedures as below.

	VAC055	VAC059	EBL01
Travel expenses	£6	£10	£10
Inconvenience of blood tests	£6	£10	£10
Time required for visit:	£15/hour	£20/hour	£20/hour
Illness Compensation	£360	£480	N/A

Table 4: Compensation rates

The variation in compensation rates was a reflection of inflation and living costs over time, and harmonisation with rates from other local trial units. The total estimated compensation per volunteer in each trial was variable and dependent on number of trial visits completed, number of additional trial visits and blood tests necessary, and whether or not they completed a rechallenge. Where travel expenses were greater than £10 per visit because the volunteer lives outside the city of the trial site, the volunteer could request further reimbursement to meet the cost of travel necessary for study visits at the investigator's discretion.

2.3.7. Exclusion criteria at the time of vaccination

The following adverse events constituted contraindications to administration of vaccine at that point in time; if any one of these adverse events occurred at the time scheduled for vaccination, the subject was vaccinated at a later date (withdrawal at the discretion of the investigator was also an option)

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever.) Some vaccines were administered to persons with a minor illness (eg. diarrhoea, mild upper respiratory infection etc.)
- Temperature of ≥37.5°C (99.5°F) at the time of vaccination.

2.3.8. Exclusion criteria on Day of CHMI (VAC055 & VAC059 only)

The following constituted absolute contraindications to CHMI;

- Acute disease, defined as moderate or severe illness with or without fever.
- Pregnancy.

2.3.9. Withdrawal of Volunteers

In accordance with the principles of the contemporary revision of the Declaration of Helsinki (updated 2008) and any other applicable regulations, a volunteer had the right to withdraw from the study at any time and for any reason, and was not obliged to give his or her reasons for doing so. In addition, the volunteer could withdraw/be withdrawn from further study procedures at any time in the interests of the volunteer's health and well-being, but no such withdrawals were required in any trial presented here.

The reason for withdrawal from further study procedures was recorded in the CRF. Except in case of complete consent withdrawal, long-term safety data collection, including some procedures, such as safety bloods, were continued if the volunteer had received one or more vaccine doses or undergone CHMI. For all AEs, appropriate follow-up visits or medical care was arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

2.4. Criteria for dose continuation and dose escalation

The VAC055 study was not a first-in-man study and did not have a dose escalation process. VAC059 was the first study to evaluate concomitant administration of AS01 with the ChAd63 and MVA vectored vaccines. There was a stepwise vaccination process as detailed below. The dose continuation/escalation process for EBL01 is detailed below.

2.4.1. Stepwise Vaccination Process for Concomitant Administration of RTS,S/AS01B with ChAd63 or MVA ME-TRAP

A sentinel group of 2 subjects in each of group 3 & 4 only was vaccinated on Day 0 and day 28. These subjects were reviewed at the Day 1 visit, and reactogenicity discussed with the chief investigator prior to vaccination of the remaining subjects in the group.

2.4.2. EBL01: Enrolment of Group 1 (ChAd3-EBO Z 1 x 10^{10}vp)

The first volunteer to receive a vaccine dose in group 1 was vaccinated alone and 48 hours elapsed before vaccinating subsequent volunteers in this dose group. Two further group 1 volunteers were vaccinated ≥48 hours after the first, after a review of the vaccine safety profile by the Chief Investigator (CI) and local safety monitor (LSM). A further ≥48-hour gap elapsed before vaccinating a further two subjects in group 1. Once five subjects had been vaccinated in group 1 and followed up for at least 48 hours, the remainder of group 1 were enrolled.

2.4.3. EBL01: Enrolment of group 2 (2.5 x 10^{10}vp)

Enrolment of the first volunteer in group 2 only proceeded once the CI and LSM had assessed the data from the first three vaccinees in group 1 as indicating that it was safe to do so. The first volunteer in group 2 was enrolled ≥48 hours after the third vaccinee in group 1 and following a safety review.

The first subject in group 2 was vaccinated alone, and a ≥48-hour gap elapsed before vaccinating further subjects in this group. Two further subjects in group 2 were vaccinated ≥48 hours after the first, once the CI and LSM had reviewed the safety profile of the first group 2 vaccine recipient, and assessed the data as safe to do so. A further ≥48-hour gap elapsed before vaccinating a further two subjects in group 2. Once five subjects had been vaccinated in group 2, and followed up for ≥48 hours, the remainder of group 2 were enrolled.

2.4.4. EBL01: Enrolment of group 3 (5 x 10^{10}vp)

Enrolment of the first volunteer in group 3 only proceeded once the CI and LSM had assessed the data from the first three vaccinees in group 2 as indicating that it was safe to do so. The first volunteer in group 3 was be enrolled ≥48 hours after the third vaccinee in group 2 and following a safety review.

The first subject in group 3 was vaccinated alone, and a ≥48-hour gap allowed before vaccinating further subjects in this group. Two further subjects in group 3 were vaccinated ≥48 hours after the first, once the CI and LSM had reviewed the safety profile of the first group 3 vaccine recipient, and assessed the data as safe to do so. A further ≥48-hour gap elapsed before vaccinating two further subjects in group 3. Once five subjects had been vaccinated in group 3, and followed up for ≥48 hours, the remainder of group 3 were enrolled. Once 5 subjects in group 3 had been

vaccinated, and followed up for 7 days, an interim safety review was performed by the Data Safety Monitoring Board (DSMB).

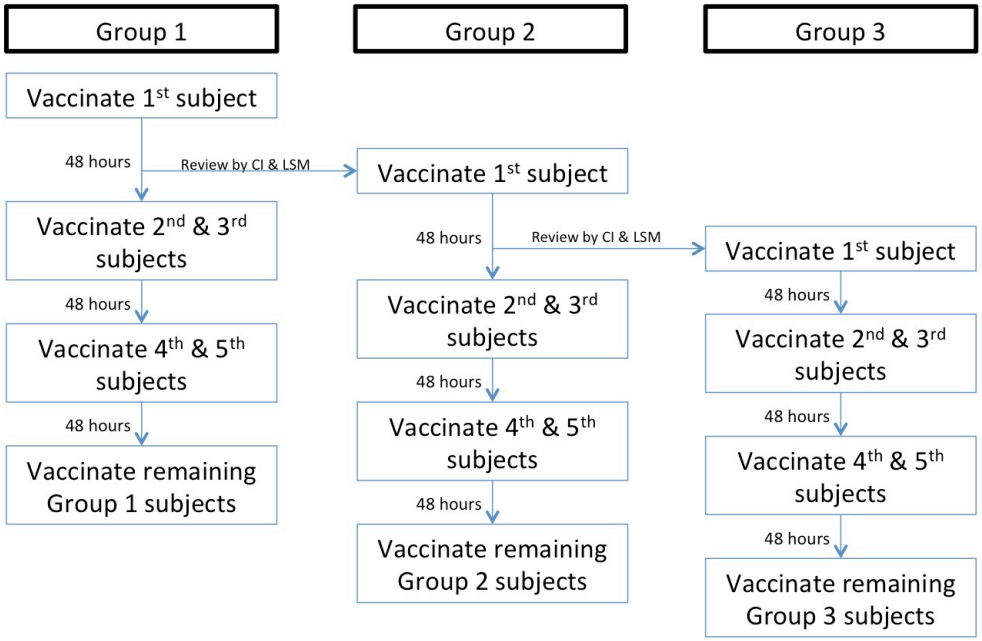


Figure 5: EBL01: Pathway for dose escalation and vaccination of subjects in Groups 1, 2 and 3

2.4.5. Initiation of vaccinations with MVA-BN® Filo in Groups 1-3

The vaccination of the first subjects to receive MVA-BN® Filo occurred in the following sequence. A single subject in one of the 3 cAd3-EBO Z dose groups was vaccinated with MVA-BN® Filo. Approximately 48 hours then elapsed, before 2 further subjects were vaccinated with MVA-BN® Filo following a safety review by the CI and LSM. Another 48 hours elapsed before the next 2 subjects were vaccinated with MVA-BN® Filo, following a safety review of the first 3 vaccinees. A further 48 hours then elapsed before all remaining volunteers in all dose groups were allowed to be vaccinated, and following a safety review by the CI and LSM.

2.4.6. EBL01: Enrolment of Groups 4-7

Preliminary analysis of the safety of both vaccines in Groups 1-3 had been completed by the time of enrolment of Groups 4-7. The majority of adverse events reported in all three dose groups of ChAd3-EBO-Z had been mild in severity with no SUSARs or SAEs at that point. Similarly, the majority of adverse events post- MVA-BN® Filo had been mild in maximal severity and there were no SAEs or SUSARs at that point. Therefore, no staggered enrolment was employed for Groups 4-7.

2.5. Safety stopping and holding rules

In addition to the dose continuation and dose escalation criteria, and the criteria for exclusion at the time of vaccination, individual and group stopping and holding rules existed for each trial. These were based on specific frequencies, severities and durations of adverse events, or the occurrence of serious adverse events related to vaccination. No stopping and holding rules were activated in any trial presented here.

2.6. Treatment of Trial Volunteers

2.6.1. Trial Sites

Volunteers were recruited and underwent screening visits, vaccination and clinic visits post vaccination at their local trial site; either CCVTM, Oxford (**all trials**), WTCRF, Southampton (**VAC055 & VAC059 only**), Surrey Clinical Research Centre, Guildford (**VAC059 only**) or the Hammersmith Hospital NIHR Wellcome Trust CRF London (**VAC055 & VAC059 only**). All volunteers travelled to Imperial College, London to undergo CHMI by mosquito bite (**VAC055 & VAC059 only**). From days 6.5 until completion of a curative course of anti-malarial therapy, all volunteers resided

in Oxford and attended follow-up visits at the CCVTM, Oxford. Southampton, Surrey and London volunteers were provided with accommodation in Oxford for this period (**VAC055 & VAC059 only**).

2.6.2. Study Procedures

For each trial, vaccinations were performed according to the study design followed by a series of safety follow up visits. Procedures were performed at the time points indicated in the relevant schedule of procedures for each trial. Schedules of procedures can be found in the supplementary appendix Table 41 to Table 50. Additional procedures or laboratory tests were performed, at the discretion of the investigators if clinically necessary. Procedures for the CHMI are detailed in section 2.7.

2.6.3. Screening Visit

All potential volunteers attended a screening visit, which took place no more than 90 days prior to enrolment. In some cases, the initial screening visit occurred more than 90 days prior to enrolment. In these cases, volunteers were required to attend a repeat screening. Informed consent was given before screening as described above.

Abnormal clinical findings from the medical history, physical examination or blood tests at any point in the study were assessed. If a test was deemed clinically significant it may have been repeated to ensure it was not a single occurrence. If an abnormal finding was deemed to be clinically significant, the volunteer would be

informed, excluded if necessary and appropriate medical care arranged with the permission of the volunteer.

2.6.4. Blood Tests, oral fluid tests, urinalysis and electrocardiograms

Blood was drawn at the time points indicated in the schedule of procedures (See appendix) and the following laboratory assays were performed;

At Oxford University Hospitals' NHS Trust, Southampton General Hospital, Frimley Park Hospital NHS Foundation Trust, or Hammersmith Hospital using NHS standard procedures

- **Haematology;** Full Blood Count
- **Biochemistry;** Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Cholesterol
- **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (Counselling will be given prior to testing blood for these blood-borne viruses)
- **Immunology;** Human Leukocyte Antigen (HLA) typing.

At the Jenner Institute research laboratories

- **Diagnostic Tests:** Blood films for malaria parasites, PCR for *P. falciparum* DNA and anti-schizont antibodies. (**VAC055 & VAC059 only**)
- **Immunology:** *Ex vivo* ELISPOT assays for interferon gamma and flow cytometry assays were performed. Other immunological assays including cytokine analysis, antibody assays, anti-adenovirus antibodies, DNA and RNA analysis of genetic polymorphisms potentially relevant to vaccine

immunogenicity and gene expression studies amongst others were also performed at the discretion of the investigators.

- **Oral fluid (EBL01 only);** Oral fluid was collected at several time points specified for exploratory immunological tests, including detection of Ebola specific antibodies.

At collaborating Laboratories for Immunomonitoring & Harmonisation

- Samples were sent to collaborating labs internationally for other immunological assays
- **Urinalysis:** Urine was tested for the presence of clinically significant proteinuria, glucosuria or haematuria at screening and at various follow-up time points. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β HCG) at screening, prior to each vaccination, the day before CHMI, and immediately before anti-malarial therapy is started.
- **Electrocardiogram (VAC055 & VAC059 only):** An electrocardiogram was performed at the initial screening visit. For Group 1 or 2 volunteers in VAC055 who underwent re-challenge had a repeat ECG as part of re-screening prior to re-challenge.

2.6.5. Administration of Vaccines

2.6.5.18. RTS,S/AS01B Injection

Intramuscular injections of RTS,S/AS01B were administered in the deltoid muscle of the left arm. The study vaccines were never administered intravascularly or intradermally. The needle used was long enough to reach the muscle mass and

prevent vaccine from seeping into subcutaneous tissue, but not so long as to involve underlying nerves, blood vessels or bone. An individual decision on needle size and site of injection must be made for each person on the basis of age, and the size of the muscle. Firm pressure was applied to the injection site (without rubbing) for at least two minutes.

RTS,S/AS01B was supplied such that the reconstituted vaccine volume provided a 0.5 mL dose for injection.

The top of vaccine vial (pellet) and adjuvant vial was disinfected with alcohol swabs and let dry. The content of the adjuvant vial was withdrawn in a syringe and the adjuvant injected into the vial of lyophilized antigen. The pellet was then dissolved by gently shaking the vial. At least 1 minute must then elapse to ensure complete dissolution of vial content before the contents of the vial (0.5 mL) was withdrawn. The needle was changed before injection.

RTS,S/AS01B was injected within 4 hours of reconstitution (storage at +2°C to +8°C/36°F to 46°F).

2.6.5.19. Administration of Viral Vectored Vaccines (ChAd63 ME-TRAP, MVA ME-TRAP, ChAd3-EBO Z & MVA-BN® Filo)

All vaccinations were administered intramuscularly according to local SOPs. Vaccinations were administered within 1 hour of thawing of the viral vectored vaccines. Vaccines were kept at room temperature between removal from the refrigerator or freezer and administration.

The vaccinating investigator wore gloves and eye protection. During administration of the vaccines, Advanced Life Support drugs and resuscitation equipment were immediately available in case of anaphylaxis.

2.6.5.20. Specific procedure for concomitant RTS,S/AS01B & viral vector vaccinations (VAC059)

For those volunteers who received concomitant administration of vaccines in VAC059, RTS,S/AS01B vaccination always preceded viral vector vaccination. Both vaccines were prepared for administration as above. Prior to cleaning the skin over the administration site, a circular area of approximately 1 centimetre diameter was drawn with a skin marker. Cleaning of the site was performed as per local SOPs, followed by administration of RTS,S/AS01B using the drawn circle as a surface marker for skin puncture. Immediately after administration of RTS,S/AS01B the viral vectored vaccine was given, again using the circle as a marker for skin puncture. Both injections were given within the 1 cm circle. The interval between vaccinations was no longer than 5 minutes. Following viral vectored vaccination firm pressure was applied to the injection site (without rubbing) for at least two minutes.

2.6.5.21. Minimising Environmental Contamination with Genetically Modified Organisms (GMOs)

These studies were performed in accordance with UK Genetically Modified Organisms (GMO) (Contained Use) Regulations (2000). In order to minimise dissemination of the recombinant vectored vaccine viruses into the environment,

inoculation sites were covered with a dressing after immunisation. This should have absorbed any virus that may have leaked out through the needle track. The dressing was removed from the injection site after ≥30 minutes (+/- 5 minutes) and was disposed as GMO waste by autoclaving.

2.6.6. Day of Vaccination

Before each vaccination, the on-going eligibility of each volunteer was reviewed. The vaccine will be administered as described in section 2.6.5. The injection site was covered with a sterile dressing and the volunteer will stay in the clinical area for 30 minutes post vaccination at which point the sterile dressing was removed and injection site inspected. Each volunteer was given an oral thermometer, tape measure and access to a 7-day electronic or paper diary card for solicited AEs.

Diary cards collected information on the timing and severity of the following solicited AEs:

Local solicited AEs	Systemic solicited AEs
Pain	Fever
Redness	Feverishness/Chills
Warmth	Joint pains
Swelling	Muscle pains
Itch	Fatigue
	Headache
	Malaise

Table 5: Solicited AEs as collected on post vaccination diary cards

Volunteers were instructed on how to self-assess the severity of these AEs. There was also space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

2.6.7. Clinical Reviews

The procedures included in each clinical review visit are documented in the schedule of procedures specific to each trial. These can be found in the supplementary appendix. Each review was assigned a time point and a window period within which the review was conducted.

2.7. Controlled Human Malaria Infection Procedures (VAC055 & VAC059 trials only)

2.7.1. Day before CHMI (C-1)

Any new medical issues or symptoms that had arisen were assessed. Physical observations, urine β-HCG test in female volunteers and venepuncture for immunology, PCR and safety bloods were undertaken according to the schedule of attendances (supplementary appendix Table 41 to Table 50). Results of safety bloods taken at this visit were reviewed prior to CHMI.

2.7.2. Day of CHMI (C0)

A Medic-Alert type card was issued to each volunteer with information including Malarone/Riamet®/chloroquine sensitivity of the challenge malaria strain, study

physician contact details and a request that the research team be contacted immediately in the event of illness/accident. Each subject was issued with an accurate oral thermometer. Volunteers were counselled about the importance of keeping mobile phones switched on and checking the messages regularly. In addition, full contact details for each subject were documented, including home address, home and work land-line telephone numbers where available, and next-of-kin address and telephone numbers. Volunteers were also required to provide the investigators with the name and 24-hour telephone number of a close friend, relative or housemate who would be kept informed of their whereabouts for the duration of the study.

2.7.3. CHMI Procedure

Anopheles stephensi mosquitoes, from the Walter Reed Army Institute of Research (WRAIR), Maryland, USA, were infected via a membrane feeder containing parasite material (fully sensitive 3D7 *P. falciparum*) plus fresh red cells and AB human serum. The blood products came from volunteers who are recruited through WRAIR and who had been screened according to US Red Cross guidelines for *Trypanosoma cruzi*, HIV 1 and 2 , HBV, HCV, HTLV, West Nile Virus and syphilis. Challenge of volunteers by infected mosquitoes was carried out in the designated containment level 3 (CL3) suite within the Sir Alexander Fleming Building at Imperial College, London. Mosquitoes were supplied directly from a representative of WRAIR, whereupon an appropriate researcher (fully trained and inducted according to local rules) from Imperial College acknowledged receipt of the mosquitoes. Infected mosquitoes were immediately transferred to a secure room within the insectary. Each pot of

mosquitoes was kept in a strong transparent plastic storage box, or within a secure Perspex glove box. All manipulation of mosquitoes was performed within a secure Perspex glovebox cabinet. Prior to challenge, an appropriately trained researcher from Imperial College prepared the mosquitoes for challenge as described. The small pots of mosquitoes brought out for challenge of the volunteers were covered with netting and held in a second pot to minimise any possibility of escape. The challenge was performed in a part of the secure insectary that is isolated by appropriate and approved mechanisms**.**

Prior to challenge, all volunteers were briefed regarding Imperial College local safety rules. Healthy human volunteers were exposed to the bites of five infectious mosquitoes per participant (by placing their forearms over the pot containing mosquitoes) for 5-10 minutes. Fed mosquitoes (as indicated by the presence of a blood meal in the abdomen) were individually dissected and assessed for sporozoite load (graded 0 to +4; a gland rating of +2 or more, representing 10 or more observed sporozoites, qualifies as being infectious). If, by this method the volunteer was found to have been inoculated by less than 5 infected mosquitoes, further mosquitoes were allowed to feed on the volunteer until a total of 5 appropriately infected mosquitoes had fed. The bite-challenge procedure continued until the subject has been bitten by 5 infectious mosquitoes (102–104 sporozoites per salivary gland). The CHMI procedure took place over 2 days with equal numbers of vaccinees and controls exposed each day.

2.7.4. Days 1-5 post CHMI (C1 - C5)

From days 1-5 post mosquito bite, volunteers returned to their own homes in either Oxford, Southampton, Surrey or London. Volunteers were phoned daily during this period to make sure they are well and contactable

2.7.5. Days 6.5 – Day 23 (VAC055) or Day 25 (VAC059) Post CHMI (C6.5 – C23/C25)

Follow-up visits of all volunteers in this phase took place at the CCVTM in Oxford. Monitoring took place twice daily by clinical review and using Giemsa-stained thick blood films, which were considered positive if a single morphologically correct parasite was seen, and by quantitative PCR starting on day 6.5 until day 15. Monitoring was then reduced to once daily until the end of the study period and completion of malaria treatment (C+23 for VAC055, C+25 for VAC059). Subjects were treated with Riamet after the first confirmed positive blood film or at the end of the monitoring period (C+21 for VAC055, C+23 for VAC059) if no parasitaemia was detected. Subjects were encouraged to contact one of the investigators on the 24 hour emergency mobile telephone number if they developed symptoms of malaria or concerning AEs between the regular clinic reviews. In addition to vaccinated subjects unvaccinated subjects were infected with malaria as infectivity controls. We reviewed volunteers at 35, 90 and 150 days following challenge for safety and immunology assessment.

2.7.6. Malaria Diagnosis

Diagnosis of malaria infection following challenge was defined as positive thick film microscopy (at least one morphologically normal malaria trophozoite seen in 200 high-power (1000x) fields) by one or more experienced microscopists in a patient with symptoms suggestive of malaria. Real time quantitative PCR for *P. falciparum* was simultaneously performed, although Investigators (except the Chief Investigator) were blinded to the results. If a positive thick film for malaria parasites was seen in an asymptomatic volunteer, the most recent PCR results for this volunteer were un-blinded and the volunteer treated only if any available PCR result for that individual had been measured as >500 parasites/ml. In this scenario, if all available PCR results were <500 parasites/ml, treatment was delayed until either the patient developed a further positive thick film in the presence of symptoms suggestive of malaria infection in the opinion of the Investigator, or if the volunteer had a further positive thick film with a PCR measurement above 500 parasites/ml (Table 6).

MALARIAL SYMPTOMS	**THICK FILM MICROSCOPY**	
	Positive	**Negative**
Symptomatic	Successfully Infected	Successfully infected if any available PCR result is > 500 parasites/ml
Asymptomatic	Successfully infected if any available PCR result is > 500 parasites/ml (Otherwise delay treatment)	Not infected

Table 6: Malarial Diagnosis for Primary Endpoint Evaluation

2.7.7. Malaria Parasite Quantitative PCR (qPCR – VAC055 & VAC059 only)

P. falciparum qPCR was performed in the Jenner Institute research laboratories, Oxford, UK by a team led by Nick Edwards. as previously described.[80] Blood was collected at baseline and at time points following CHMI for qPCR in 2.0ml tubes containing EDTA before being filtered to reduce white cell content. DNA was extracted from 0.5mL filtered blood using Qiagen Blood Mini Kit. 5µL of each extraction was used per assay (total eluate volume = 50µL) and was run in triplicate for qPCR (equivalent to 150µL blood directly assessed). Parasites per mL (p/mL) equivalent mean values were generated by a standard Taqman absolute quantitation, against a defined plasmid standard curve. This was conducted on an ABI StepOne Plus machine and v2.3 software using default Universal qPCR and QC settings, apart from the use of 45 cycles and 25µL reaction volume.

2.7.7.22. Parasite Growth Modelling (VAC055)

To analyse parasite density using qPCR, mean and standard deviation (SD) were calculated within each treatment group at cycle peaks (C+7.5, C+9.5 and C+11.5). For each subject, the area under the curve (AUC) was calculated between first and third cycles; which was also summarized within treatment groups (mean and SD). The fold rise from cycle 1 peak was calculated at cycles 2 and 3, which was then summarized within treatment groups (mean and SD). These three characteristics of parasite density were compared at each time point.

2.7.8. Malaria Management

Volunteers were treated with a standard treatment dose of oral Riamet (artemether/lumefantrine 20/120mg) on diagnosis. Volunteers were reminded of the potential side effects and given the patient information sheet for Riamet and a card outlining when their doses of Riamet should be taken. For subjects with contraindications to Riamet, or who were intolerant of treatment, an alternative oral antimalarial (Malarone or chloroquine) was dispensed.

Volunteers who remained undiagnosed with malaria at the end of monitoring (C+21 for VAC055, C+23 for VAC059) were started on a treatment course of Riamet at this visit. At least half of the Riamet doses for each volunteer were observed in clinic by the study team. (For volunteers who took chloroquine, the same applied, and for volunteers who took malarone, all doses were observed).

2.7.9. Follow Up Post Diagnosis

Subjects were reviewed in clinic approximately 24 and 48 hours after diagnosis (and the start of anti-malarial therapy), when physical observations, assessment of symptoms and venepuncture were performed (supplementary appendix Table 42 & Table **43**, Table 45 &Table **46**). Volunteers continued to be reviewed in clinic daily until they had 2 consecutive negative blood films at least 24 hours apart following start of antimalarial treatment, and all symptoms were mild or resolving. Volunteers were given a card on which to document the end of date of any outstanding malaria symptoms on-going between completing anti-malarial therapy and the next study visit (C+35).

2.7.10. Post CHMI visits (C+35 onwards)

Further visits and procedures after the completion of malaria treatment will be conducted as outlined in the schedule of attendances (appendix).

2.7.11. Re-Challenge of Protected Vaccinees (VAC055 only)

Group 1 and 2 volunteers who were sterilely protected following initial CHMI were invited to undergo re-challenge 6 months after the first challenge in order to assess the durability of vaccine protection. Volunteers who agreed to undergo repeat CHMI received a re-challenge volunteer information sheet, and were required to undergo a re-screening visit to ensure that they remained eligible and to recheck screening blood tests, perform a repeat ECG, discuss the details of the repeat CHMI and re-sign a written informed consent form. The re-challenge followed the same course and study procedures as the initial CHMI. New unvaccinated volunteers were recruited to act as infectivity controls for the repeat CHMI.

2.8. Assessment of Safety

Safety of the investigational medicinal products (IMP) was assessed by analysing the frequency, incidence and nature of adverse events and serious adverse events arising during each study. Each adverse event was graded by the participant according to the table for grading severity of adverse events (see Table 8, Table 9, Table 10). Severity gradings were reviewed and discussed with the participants at the clinic visits.

Solicited adverse events (i.e, reactogenicity parameters) were recorded on diary cards (paper or electronic) for 7 days after vaccination without the collection of attribution assessments. Unsolicited AEs of all severities were recorded on diary cards (paper or electronic) from receipt of vaccination through 28 days post-vaccination (**VAC059 & EBL01**) or 30 days post vaccination (**VAC055**). After Study Day 28 (**VAC059 & EBL01**) or 30 (**VAC055**), unsolicited AEs and SAEs (as defined below) were recorded through the last study visit on the study CRF.

2.8.1. Definitions

Adverse Event (AE): An AE was any untoward medical occurrence in a volunteer, including dosing errors, which occurred during or after administration of an IMP and does not necessarily have a causal relationship with the intervention. An AE could therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Adverse Reaction (AR): An AR was any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by either the reporting medical investigator or the sponsors as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

Unexpected Adverse Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (*e.g.*, Investigator's

Brochure for an unapproved investigational medicinal product) is considered as an unexpected adverse drug reaction.

Serious Adverse Event (SAE): An SAE was an AE that resulted in any of the following outcomes, whether or not considered related to the study intervention.

- Death (i.e., results in death from any cause at any time)
- Life-threatening event (i.e., the volunteer was, in the view of the investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.

- Congenital anomaly or birth defect.

Serious Adverse Reaction (SAR): An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting investigator or sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided.

Suspected Unexpected Serious Adverse Reactions (SUSARs): A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely related to an IMP.

2.8.2. Causality Assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine was undertaken. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question was made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy (Table 7) Causality assessment took place during interim and final safety analyses.

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 7: Guidelines for assessing the relationship of vaccine administration to an AE

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	>0 - ≤20 mm**
	2	>20 - ≤50 mm
	3	>50 mm

Table 8: Severity grading criterion for injection site pain, erythema and swelling. *erythema ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event. **Grade 1 swelling in EBL01 was defined as >1 - ≤20mm

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible

Table 9: Severity grading criterion for AEs.

	Grade 1 (mild)	**Grade 2 (moderate)**	**Grade 3 (severe)**
Fever (oral)	37.6°C - 38.0°C	38.1°C – 39.0°C	>39.0°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130
Bradycardia (bpm)**	50 – 54	40 – 49	<40
Systolic hypertension (mmHg)	141 - 159	160 – 179	≥180
Diastolic hypertension (mmHg)	91 - 99	100 – 109	≥110
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80

Table 10: Severity grading criteria for physical observations; *Taken after ≥10 minutes at rest; **When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes; ***Only if symptomatic (e.g. dizzy/ light-headed). *Systolic or diastolic hypertension may only be confirmed as clinically significant (and therefore an AE) if persistently present when observations are repeated (i.e. isolated measurements of hypertension are not clinically significant).*

2.8.3. Additional Safety Oversight

A Local Safety Committee (LSC - **VAC055 & VAC059 only**) or Data Safety Monitoring Board (DSMB – **EBL01 only**) was appointed for each trial to provide real-time safety oversight.

2.9. Immunological Assays

Immunological assays including *ex-vivo* Interferon-gamma (IFN-γ) enzyme-Linked Immunosorbent Spot (ELISPOT), flow cytometry with intracellular cytokine staining (ICS), Enzyme Linked Immunosorbent Assays (ELISA) and neutralizing antibody (nAb) assays against live Ebolavirus were performed by teams at the Jenner Institute Research Laboratories and collaborating laboratories internationally. Specific details on the methods and the sites/individuals who performed these assays can be found in the supplementary appendix and the authorship statements preceding each chapter.

Chapter 3

Safety, Efficacy and Immunogenicity of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP (VAC055)

3. Safety, Efficacy and Immunogenicity of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP (VAC055)

3.1. Authorship Statement

The work presented in this Chapter is part of a collaborative effort. Unless otherwise stated, all work was undertaken by me.

The study was designed in collaboration with staff at GlaxoSmithkline and PATH MVI. The conduct of the trial was greatly assisted by the Jenner Institute Clinical Trials team (including Alison Lawrie, Rachel Roberts, Ian Poulton, Ruth Payne, Eoghan de Barra, Navin Venkatraman, Mary Smith, Raquel Lopez Ramon) and the teams at Southampton University Hospitals NIHR Wellcome Trust Clinical Research Facility (including Saul Faust) and Imperial College London (including Robert Sinden and Graham Cooke). This included some assistance with regulatory processes and clinical procedures (volunteer screening, follow up visits etc.).

Nick Edwards performed the qPCR. Katie Ewer, Georgina Bowyer, Carly Bliss and Danny Wright performed the immunological assays, including the ELISAs, EliSPOTs and Flow Cytometry.

Blood samples for FBC, biochemistry and serology (for HIV, Hepatitis B and C) were processed at the Trust laboratories at Oxford University Hospitals' NHS Trust, Southampton University Hospitals NHS Trust, and Imperial College NHS Trust.

Malaria films were processed by a team recruited by the Jenner Institute at the Centre for Clinical Vaccinology and Tropical Medicine.

The handling of infectious mosquitoes for CHMI (including transport, preparation for CHMI and dissection) was performed by a team lead by Robert Sinden & Jake Baum at Imperial College London.

Some of the data in this chapter has been published in the following article:

- **Rampling T**, Ewer K, Bowyer G *et al*. Safety and High Level Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01B with ChAd-MVA Vectored Vaccines Expressing ME-TRAP. J Infect Dis. 2016 Jun 15. pii: jiw244. [Epub ahead of print] PMID: 27307573

3.2. Introduction

In this phase I/IIa, open-label, CHMI study, I assessed the safety, immunogenicity and efficacy of a vaccine schedule combining two distinct candidate vaccine types in a staggered immunisation regimen: one that induces very high titre antibodies to CS using RTS,S/AS01B and the other inducing potent T cell responses to TRAP using viral vectors.

3.3. Objectives

3.3.1. Primary Objective:

To assess the efficacy (occurrence of *P. falciparum* parasitemia, assessed by blood slide) of a combination immunisation regimen with ChAd63/MVA ME-TRAP and

RTS,S/AS01B, and of RTS,S/AS01B alone, against malaria sporozoite challenge, in healthy malaria-naïve volunteers.

To assess the safety of a combination immunisation regimen with ChAd63/MVA ME-TRAP and RTS,S/AS01B, and of RTS,S/AS01B alone, in healthy malaria-naïve volunteers.

3.3.2. Secondary Objectives:

To assess immunogenicity generated in malaria naïve individuals of a malaria vaccine schedule containing RTS,S/AS01B and ChAd63/MVA ME-TRAP, and of RTS,S/AS01B alone.

To assess the efficacy (measured as time to *P. falciparum* parasitemia assessed by blood slide, by PCR, and parasite density dynamics assessed by PCR) of a combination immunisation regimen with ChAd63/MVA ME-TRAP and RTS,S/AS01B, and of RTS,S/AS01B alone, against malaria sporozoite challenge, in healthy malaria-naïve volunteers.

3.3.3. Tertiary Objectives:

To assess the long term protective efficacy of a combination vaccine schedule of RTS,S/AS01B and ChAd63-MVA ME-TRAP by re-challenge of volunteers exhibiting sterile protection.

3.4. Participants

Recruitment and vaccination was conducted at three UK sites, in Oxford, Southampton and London. The CHMI procedure was performed as described in

Chapter 2 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 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).

3.5. 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 US 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.

3.6. Study Design

This Phase IIa, open-label partially randomised challenge trial consisted of 4 cohorts. Allocation to study group occurred at screening based on subject preference. Any subjects without a preference were randomised to vaccine Group 1 or 2. Group 1 (n=20) received 5 vaccinations (RTS,S/AS01B 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/AS01B 50 µg at 0, 4 and 8 weeks); Group 3 (n=6) received no vaccinations. Study groups are summarized in Table 11. All vaccinations were administered intramuscularly (IM) into the deltoid region of the arm.

Week	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=6)	Group 4 (n=6)
0	RTS,S/AS01B	RTS,S/AS01B		
2	ChAd63 ME-TRAP			
4	RTS,S/AS01B	RTS,S/AS01B		
8	RTS,S/AS01B	RTS,S/AS01B		
10	MVA ME-TRAP			
12	CHMI	CHMI	CHMI	
32-40	Repeat CHMI of sterilely protected volunteers	Repeat CHMI of sterilely protected volunteers		CHMI

Table 11: VAC055 Clinical Trial Design

In each volunteer, all RTS,S/AS01B injections were given in one arm, and all viral vector injections were given in the contralateral arm.

Following vaccination, subjects attended a schedule of follow up visits for assessment of vaccine safety, and blood sampling for immunogenicity analysis. The full schedule of follow up visits, with details of assessments conducted at each timepoint can be found in the appendix. 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.[80] 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 Chapter 2: Materials and Methods.

3.7. Results

3.7.1. Participants

Recruitment took place between June 2013 and May 2014. Eighty subjects were screened for eligibility and 48 subjects were identified as eligible and recruited. 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 subjects in case of last minute withdrawals from Group 3, but never underwent CHMI. Seventeen subjects expressed a preference as to which vaccine group to be allocated to, and were assigned accordingly. Twenty subjects expressed no preference for vaccine group allocation, and were therefore randomised to group by the study statistician. Twenty subjects were allocated to group 1 to receive RTS,S/AS01B and viral vectors encoding ME-TRAP. Seventeen subjects were allocated to group 2 to receive RTS,S/AS01B 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 rules were activated. CHMI was performed on the 25th and 26th of November 2013, and repeat CHMI on 13th May 2014. A flow diagram of recruitment can be found in Figure 6. Demographics for the subjects enrolled are shown in Table 12.

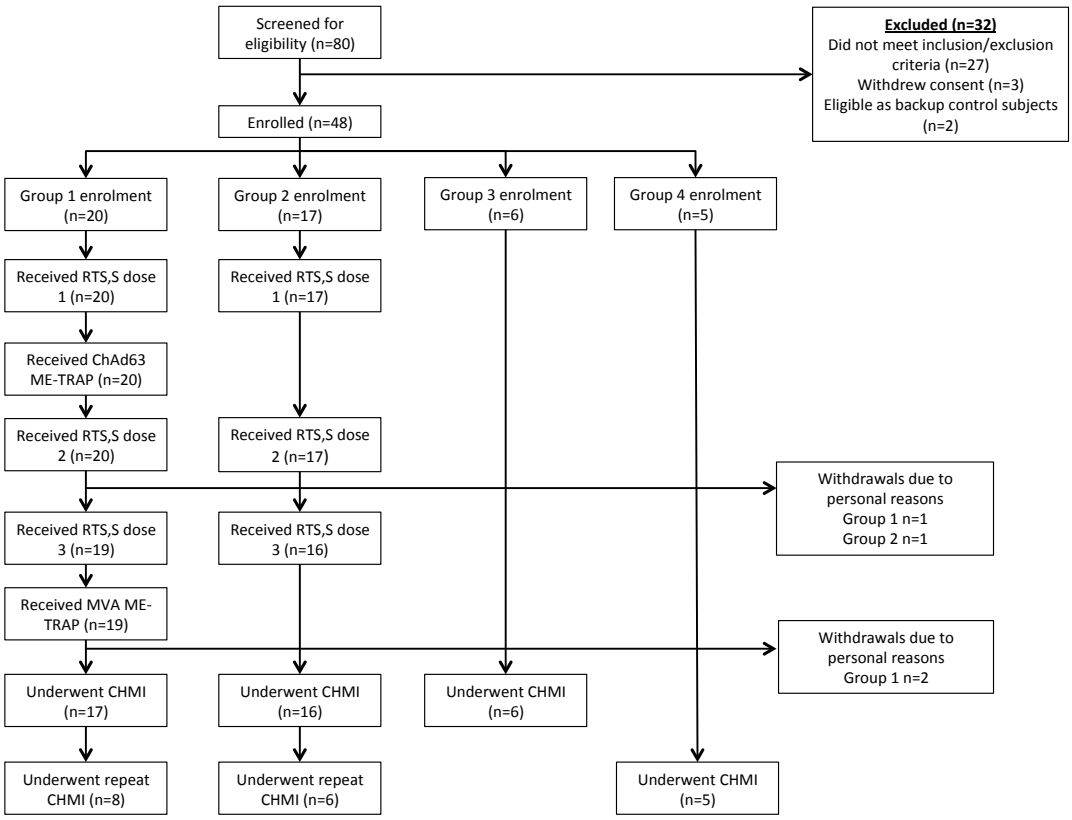


Figure 6: Flow of study design and volunteer recruitment.

	Group 1 (n=20)	Group 2 (n=17)	Group 3 (n=6)	Group 4 (n=5)	All Groups (n=48)
Gender					
Male	11 (55.0%)	9 (52.9%)	2 (33.3%)	2 (40.0%)	24 (50.0%)
Female	9 (45.0%)	8 (47.1%)	4 (66.7%)	3 (60.0%)	24 (50.0%)
Country of Birth					
UK	14 (70.0%)	13 (76.5%)	3 (50.0%)	4 (80.0%)	34 (70.8%)
Other	6 (30.0%)	4 (23.5%)	3 (50.0%)	1 (20.0%)	14 (29.2%)
Age					
Range	20-45	18-45	20-30	22-33	18-45
18-20	1 (5.0%)	3 (17.6%)	1 (16.7%)	0	5 (10.4%)
21-30	9 (45.0%)	6 (35.3%)	5 (83.3%)	4 (80.0%)	24 (50.0%)
31-40	8 (40.0%)	4 (23.5%)	0	1 (20.0%)	13 (27.1%)
>41	2 (10.0%)	4 (23.5%)	0	0	6 (12.5%)
Mean (+/- SD)	32.4 (+/- 7.36)	31.6 (+/- 9.09)	24.5 (+/- 3.88)	25.0 (+/- 4.95)	30.3 (+/- 7.96)

Table 12: Demographics of all subjects enrolled in VAC055

3.7.2. Protective efficacy against CHMI

Efficacy was assessed by CHMI with *P. falciparum* by mosquito bite, and durability of efficacy was assessed by repeat CHMI 6 months later. 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). CHMI was conducted over 2 days.

3.7.2.23. Sterile efficacy

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. Comparison by Fisher's Exact test of the primary outcome measure can be found in Table 13.

	Proportion protected
Group 1	14/17 (82.4%)
Group 2	12/16 (75.0%)
Group 3	0/6 (0%)
	Fisher's Exact test *p*
Group 1 vs. Group 3	0.0008
Group 2 vs. Group 3	0.0028
Group 1 vs. Group 2	0.6880

Table 13: Proportion of subjects completely protected by day 21 after CHMI and two-tailed Fisher's exact test.

Both vaccine regimens demonstrated significantly reduced risk of malaria infection over controls (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.

3.7.2.24. Secondary efficacy endpoints

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).

Figure 7 shows the time to malaria diagnosis (see Chapter 2 for diagnosis criteria) for the 2 vaccine groups (Groups 1 and 2) compared to controls (Group 3). Figure 8 shows the time to PCR positivity (>20 parasites/mL) and Figure 9 shows the time to 500 parasites/mL by PCR. A delay to each of these secondary endpoints was noted in volunteers from both vaccination groups, but this delay was greatest in Group 1.

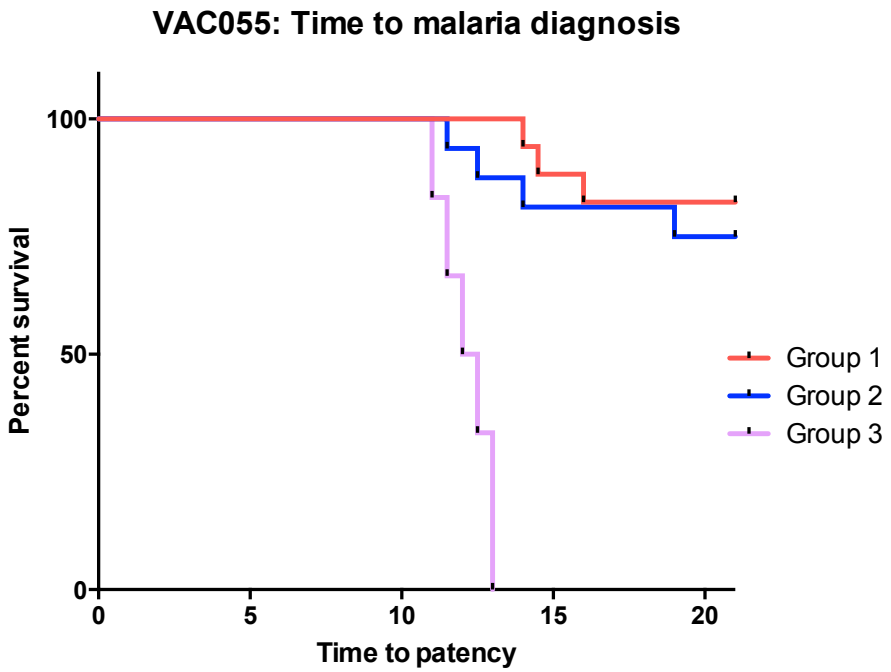


Figure 7: Kaplan-Meier survival analyses of time to malaria diagnosis (primary endpoint) by blood film and/or PCR parasitaemia >500 parasites/ml +/- symptoms in days for Group 1 vaccinees (n=17), Group 2 vaccinees (n=16) and unvaccinated infectivity controls (Group 3; n=6).

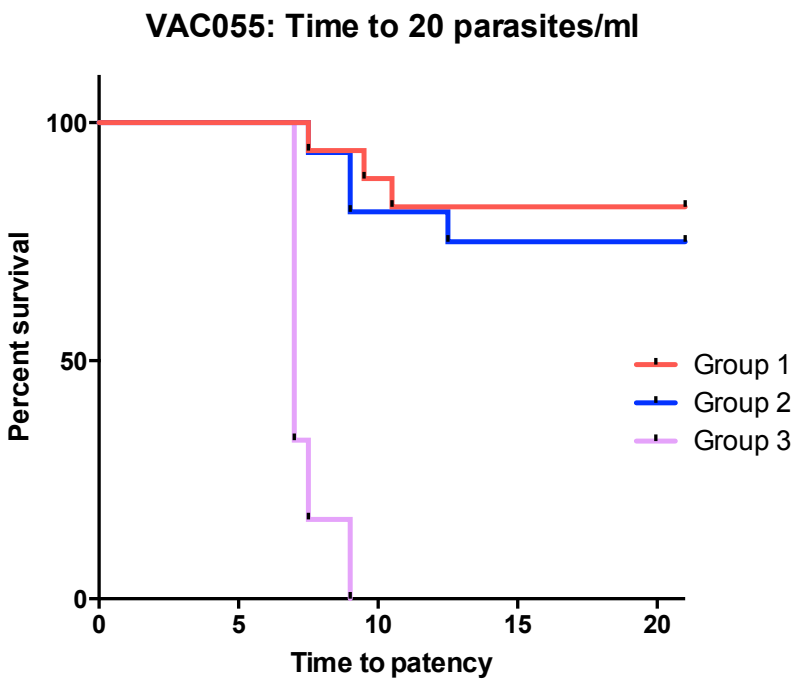


Figure 8: Kaplan-Meier survival analyses of time to 20 parasites/ml (secondary endpoint) by qPCR in days for Group 1 vaccinees (n=17), Group 2 vaccinees (n=16) and unvaccinated infectivity controls (Group 3; n=6).

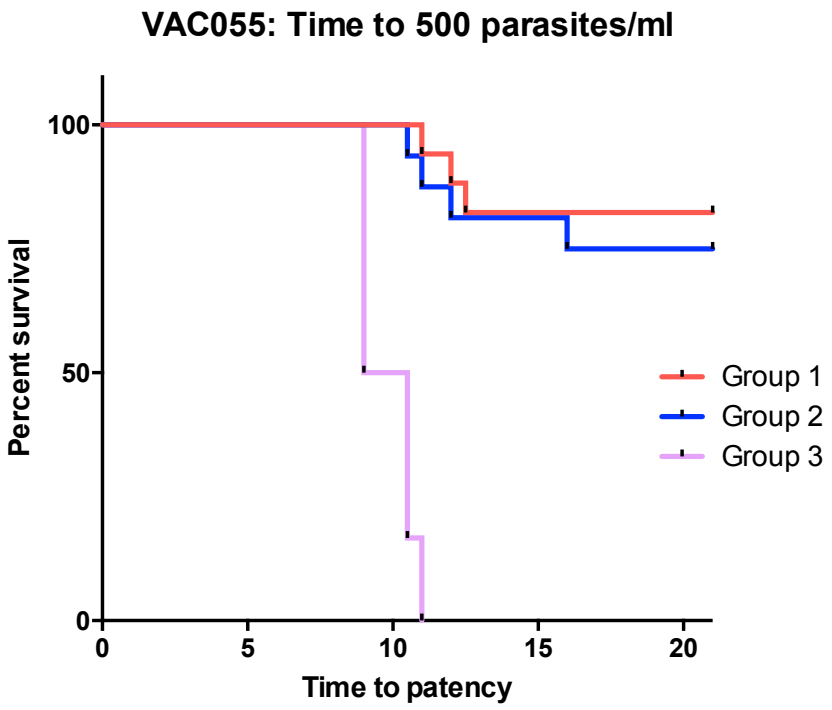


Figure 9: Kaplan-Meier survival analyses of time to 500 parasites/ml (secondary endpoint) by qPCR in days for Group 1 vaccinees (n=17), Group 2 vaccinees (n=16) and unvaccinated infectivity controls (Group 3; n=6).

	Logrank p	Hazard ratio (95% CI)
Malaria diagnosis		
Group 1 vs Group 2	0.57	0.65 (0.15-2.86)
Group 1 vs controls	<0.0001	0.065 (0.0003 – 0.019)
Group 2 vs controls	<0.0001	0.12 (0.004 – 0.13)
20 parasites/ml		
Group 1 vs Group 2	0.61	0.68 (0.15 – 2.98)
Group 1 vs controls	<0.0001	0.085 (0.0009 – 0.03)
Group 2 vs controls	<0.0001	0.118 (0.002 – 0.068)
500 parasites/ml		
Group 1 vs Group 2	0.59	0.67 (0.15 – 2.93)
Group 1 vs controls	<0.0001	0.076 (0.0006 – 0.027)
Group 2 vs controls	<0.0001	0.11 (0.0017 – 0.061)

Table 14: Secondary efficacy endpoints. Logrank test p values and hazard ratios for each of the 3 secondary efficacy endpoints.

	Control mean (SD)	Group 1 – proportion of subjects delayed	Group 2 – proportion of subjects delayed
Time to malaria diagnosis (days)	12.17 (+/- 0.7)	17/17 (100%)	14/16 (87.5%)
Time to 20 parasites/ml (days)	7.42 (+/- 0.7)	16/17 (94.1%)	15/16 (93.8%)
Time to 500 parasites/ml (days)	9.83 (+/- 0.8)	17/17 (100%)	15/16 (93.8%)

Table 15: Time to secondary efficacy endpoint. A delay to endpoint is defined here as any subject who reaches the endpoint after the control (Group 3) mean + 1 standard deviation (SD).

3.7.1. Parasite Density Endpoints:

To analyse parasite density, mean and standard deviation have been reported within each treatment group at cycle peaks (C+7.5, C+9.5 and C+11.5). For each subject, the area under the curve (AUC) was calculated between first and third cycles, which has then been summarized within treatment groups (mean and standard deviation). The mean fold rise from cycle 1 peak was calculated at cycles 2 and 3; which has again been summarised within treatment groups (Table 16).

	Group 1	Group 2	Group 3
Mean parasite density at C+7.5 (+/- SD) (p/ml)	0.8 (+/- 3.1)	1.0 (+/- 2.6)	80.3 (+/- 58.5)
Mean parasite density at C+9.5 (+/- SD) (p/ml)	5.0 (+/-13.9)	4.3 (+/- 9.9)	410.9 (+/- 279.4)
Mean parasite density at C+11.5 (+/- SD) (p/ml)	54.8 (+/- 198.7)	90.2 (+/- 325.5)	2641 (+/- 1344.5)
Mean AUC (+/- SD)	103.5 (+/- 358.7)	168.4 (+/- 465.9)	4615.9 (+/- 2177.9)
Fold rise Cycle 1-2	5.25	3.3	4.12
Fold rise Cycle 1-3	9.96	19.98	5.43

Table 16: Parasite density endpoints

There was a significant reduction of >95% in mean parasitaemia at the first 3 cycle peaks (C+7.5, C+9.5 and C+ 11.5) in both Group 1 and Group 2 when compared with controls (P<0.0001). (Figure 10). Comparing Group 1 with controls, reductions in parasite density of 100% (p<0.0001), 99.3% (p<0.0001) and 97.9% (p<0.0001) were observed on day 7.5, 9.5 and 11.5 respectively. Comparing Group 2 vs controls reductions of 97.4% (p<0.0001), 98.9% (p<0.0001) and 96.6% (p<0.0001) in mean parasite density on day 7.5, 9.5 and 11.5 respectively. P-values were determined by Mann-Whitney U-Test. In all infected subjects, parasite density at the first cycle peak (C+7.5) was negatively correlated with the time to diagnosis (Spearman R -0.576; *p*=0.039) and the area under the curve (AUC) to the 3rd cycle peak (C+11.5) in infected subjects was also negatively correlated with time to malaria diagnosis (Spearman R -0.711; p=0.009) (Figure 11). Means of log [parasite density + 1] in infected subjects were significantly different from controls in infected subjects (2-tailed t-test) in Group 1 at the first (p=0.01), second (p=0.004) and third cycles (p=0.006) and in Group 2 (p=0.02; p=0.0009 and p=0.005 at the first, second and third cycles respectively) (Figure 12).

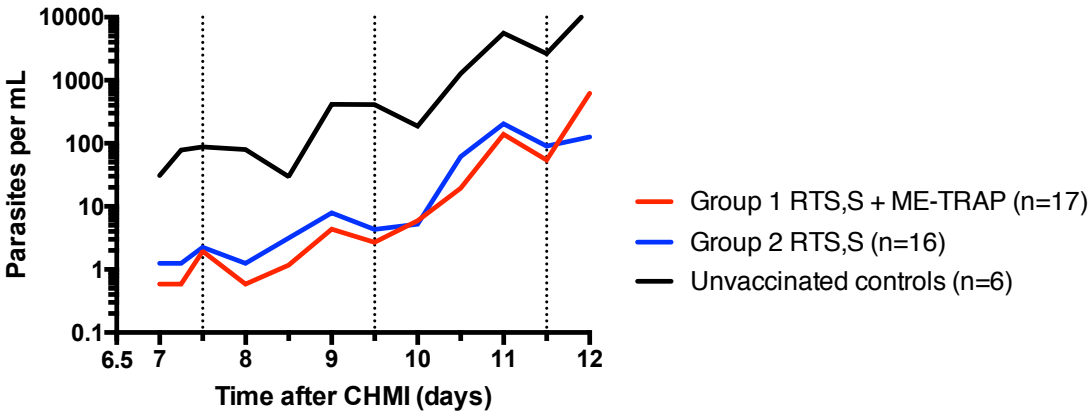


Figure 10: Comparison of mean parasite densities, measured by quantitative polymerase chain reaction at cycle peaks 7.5, 9.5, and 11.5 days after controlled human malaria infection (CHMI) between vaccinees and control volunteers

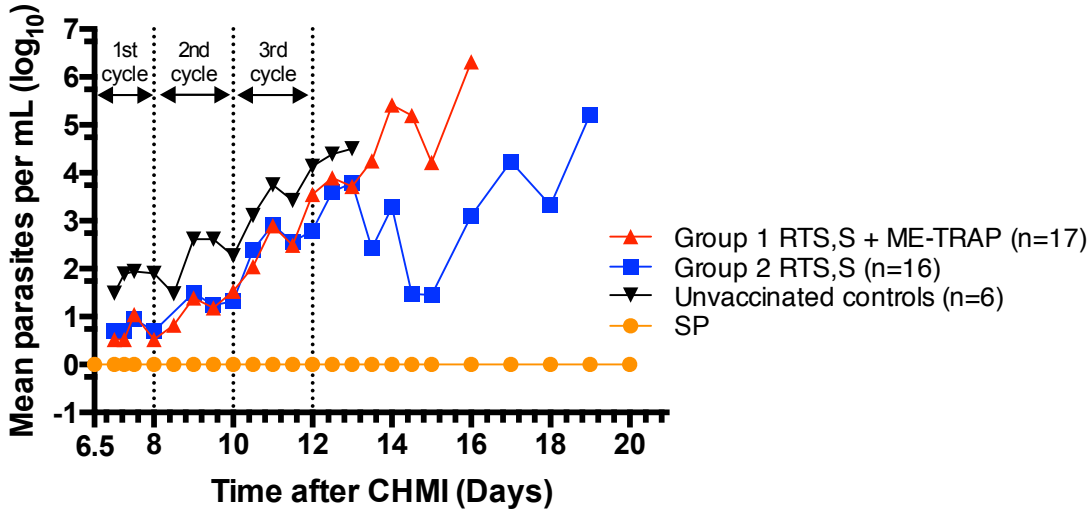


Figure 11: Group mean log-transformed PCR data in infected volunteers.

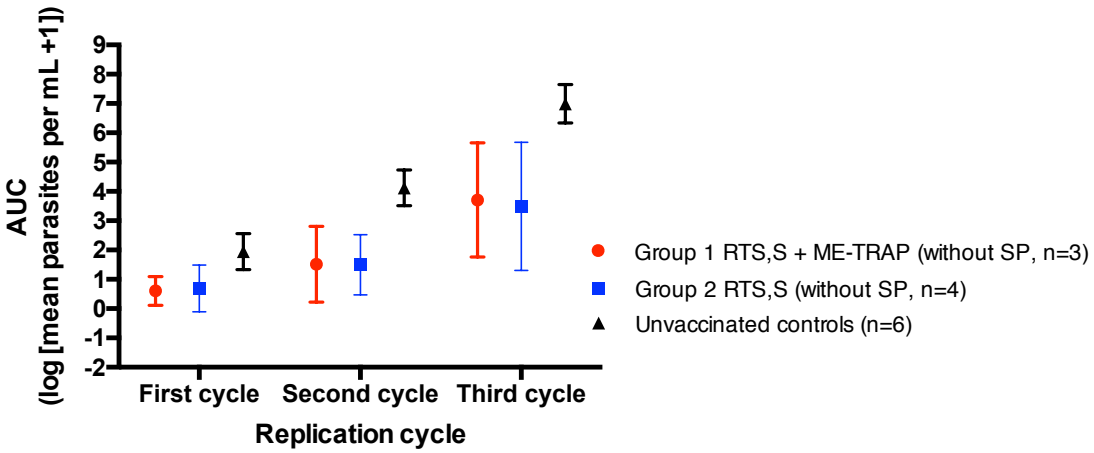


Figure 12: AUC analysis of parasite densities, comparing controls to vaccinees at days 6.5–8 (the first cycle after hepatocyte release), days 8.5–10 (the second cycle), and days 10.5–12 (the third cycle) after controlled human malaria infection (CHMI)

3.7.1.25. Tertiary efficacy endpoints

Due to the high level of protective efficacy observed in both vaccine groups, protected subjects were invited back to undergo repeat CHMI using an identical process to the primary CHMI. Of the protected subjects offered re-challenge, 8 subjects from Group 1 and 6 subjects from Group 2 agreed to participate. Repeat CHMI was conducted on the 13th May 2013. Five new unvaccinated subjects were recruited to act as infectivity controls. A single subject in each of Groups 1 and 2 was 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). The proportion of subjects protected is given in Table 17. A Kaplan-Meier plot showing time to diagnosis, and time to PCR parasitaemia can be found in Figure 13.

	Proportion protected
Group 1	7/8 (87.5%)
Group 2	5/6 (83.3%)
Group 3	0/5 (0%)

Table 17: Tertiary endpoint. Proportion of subjects protected to day 21 following repeat CHMI. Note, the figure given is the proportion of subjects who agreed to participate in the repeat CHMI.

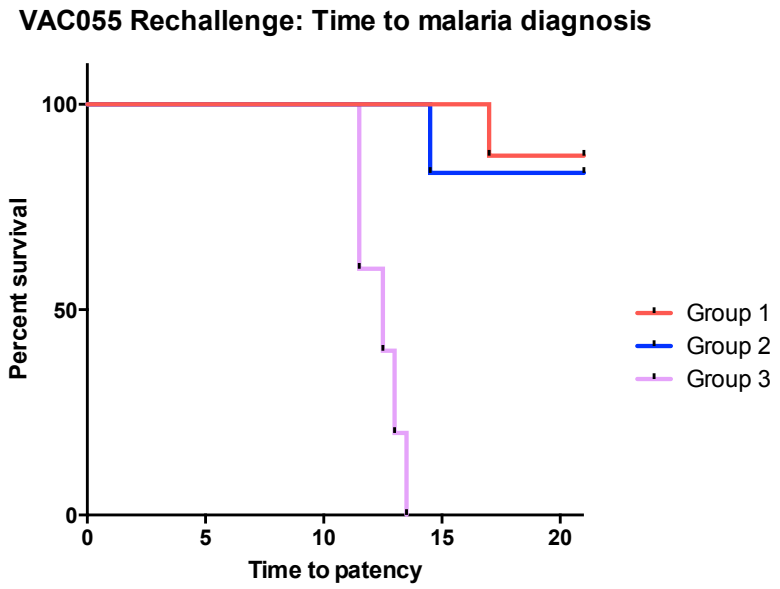


Figure 13: Kaplan-Meier survival analyses of time to malaria diagnosis following repeat CHMI at 6 months after primary CHMI by blood film and/or PCR parasitaemia >500 parasites/ml and/or symptoms in days for Group 1 vaccinees (n=8), Group 2 vaccinees (n=6) and unvaccinated infectivity controls (Group 3; n=5).

3.7.2. Safety

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.

3.7.2.26. Serious Adverse Events (SAEs)

In this trial, there were 2 SAEs and no SUSARs. Neither SAE was deemed related to vaccination, and each is described in detail below. No adverse events of special interest as defined in the protocol occurred. No pregnancies or deaths occurred.

SAE #1

On 13th March 2014 (Day 114 after CHMI and day 128 after last dose of any study IMP) this Group 1 subject twisted her ankle whilst walking on high heeled shoes. There was no preceding systemic illness or symptoms, and she reported being otherwise completely well. The following day she attended St. Mary's Hospital due to the severity of the pain, and was diagnosed with a spiral fracture of her right fibula. Her lower leg was immobilized in a cast for 6 weeks. This SAE was considered to be unrelated to study IMPs.

SAE #2

This Group 1 subject was admitted to Surrey and Sussex Healthcare NHS Trust with right-sided chest pain and shortness of breath on the 1st April 2014 (Day 126 after CHMI and day 140 after last dose of any study IMP). A VQ scan was performed which showed a low probability of pulmonary embolism. A deep vein thrombosis was excluded by doppler scan. He was noted to be visibly jaundiced, and complained of pruritus. Bilirubin was raised at 201. He was started on antibiotics for suspected biliary sepsis and started to improve. He was discharged home on the 4th April 2014

with a plan for outpatient MRCP. He is known to have chronic pancreatitis on a background of biliary stricture. This SAE was considered to be unrelated to study IMPs. He declined to have any further involvement in the study, including further follow up calls to ascertain the outcome of the planned outpatient investigations.

3.7.2.27. Adverse events following RTS,S/AS01B vaccination

The majority of adverse events following all doses of RTS,S/AS01B were mild in both vaccine groups, with occasional moderate and severe symptoms reported. There was an observed trend towards greater reactogenicity following the second and third doses of the vaccine. Adverse events were self-limiting, and no SAEs related to vaccination or SUSARs were reported. Pain was the most commonly reported adverse event after all doses of RTS,S (88.2% - 97.3% depending on dose number). Other commonly reported adverse events following RTS,S were headache (48.6% - 79.4%), fatigue (48.6% - 64.7%), feverishness (45.9% - 73.5%) and warmth (59.5% - 70.3%). The solicited adverse events reported at grade 3 (severe) intensity by 1 or more subject after 1 or more doses of RTS,S were pain, swelling, fever, feverishness, myalgia, arthralgia, headache, fatigue, nausea, malaise. Graphs of solicited adverse events following vaccination with RTS,S/AS01B can be found in Figure 14, Figure 15, and Figure 16. Full tabulation of solicited AEs by group, severity and frequency can be found in the safety appendix Table 56.

Verbatim reports of unsolicited signs and symptoms were reviewed and coded and tabulated according to the MedDRA Dictionary for Adverse Reaction Terminology (See safety appendix for full listings of unsolicited AEs). Each unsolicited AE was

annotated with a causality grading according to likely relationship to vaccination of either Unrelated, Unlikely, Possible, Probable or Definite. The percentage of subjects with at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported up to 30 days (Day 0-Day 29) after vaccination have been tabulated with exact 95% CI, and are shown in Table 18. The same tabulation has been performed for grade 3 unsolicited AEs (Table 19) and for unsolicited AEs with a relationship to vaccination (Table 20).

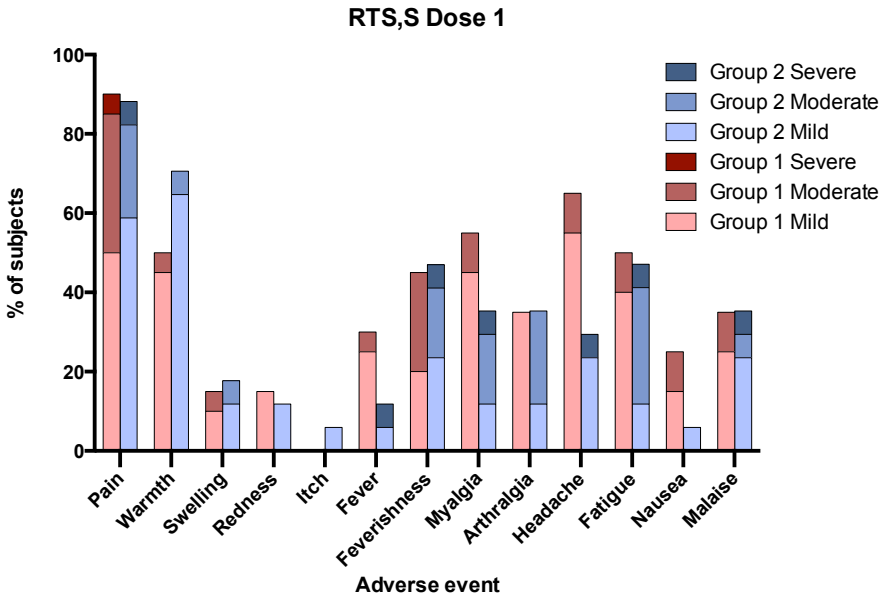


Figure 14: Solicited reactogenicity in the 7 day period following vaccination with the first dose of RTS,S/AS01B

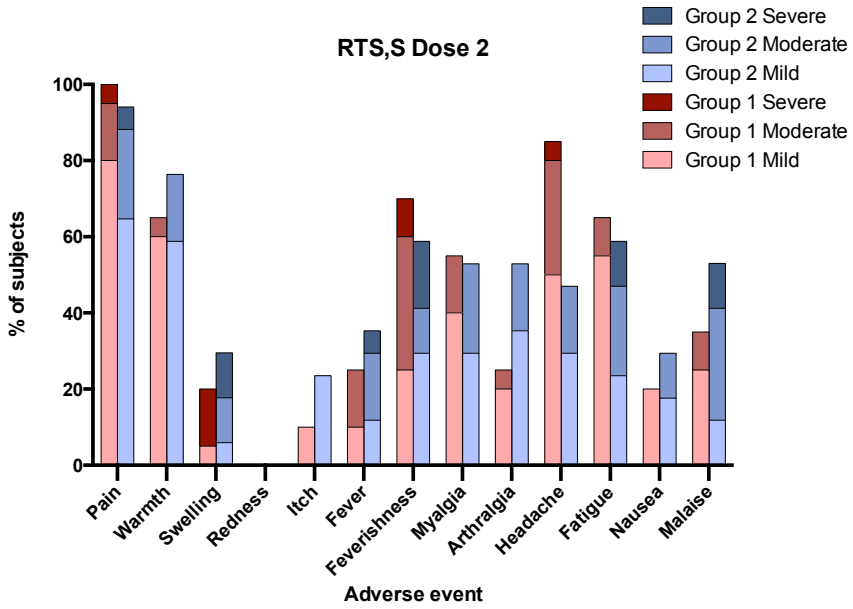


Figure 15: Solicited reactogenicity in the 7 day period following vaccination with the second dose of RTS,S/AS01B

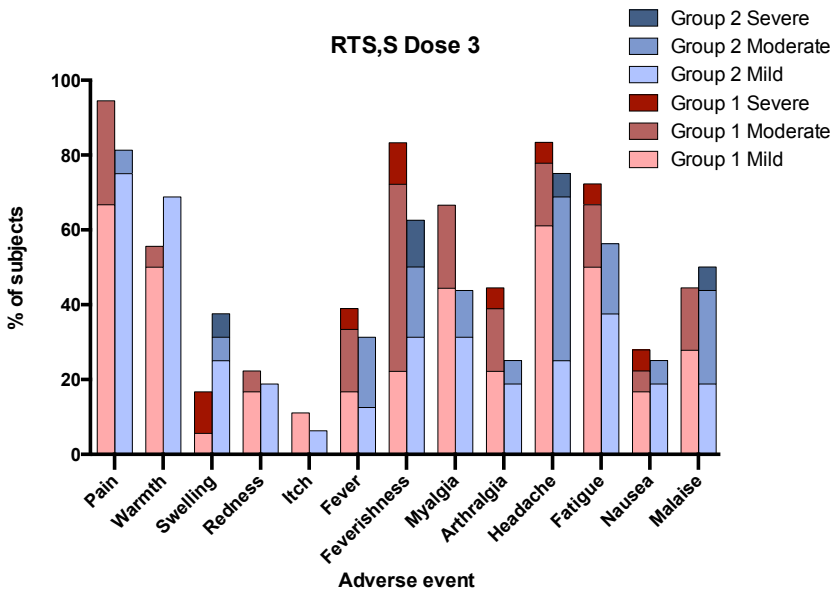


Figure 16: Solicited reactogenicity in the 7 day period following vaccination with the third dose of RTS,S/AS01B

	RTS,S/AS01B Dose 1		ChAd63 ME-TRAP		RTS,S/AS01B Dose 2		RTS,S/AS01B Dose 3		MVA ME-TRAP	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Group 1	80.0	56.3 - 94.3	75.0	50.9 - 91.3	75.0	50.9 - 91.3	66.7	41.0 - 86.7	76.5	50.1 - 93.2
Group 2	70.6	44.0 - 89.7			76.5	50.1 - 93.2	50.0	24.7 - 75.4		
Total	75.7	58.8 - 88.2	75.0	50.9 - 91.3	75.7	58.8 - 88.2	58.8	40.7 - 75.4	76.5	50.1 - 93.2

Table 18: Percentage of subjects with at least one report of unsolicited AE in the 30 day period post vaccination (Days 0-29)

	RTS,S/AS01B Dose 1		ChAd63 ME-TRAP		RTS,S/AS01B Dose 2		RTS,S/AS01B Dose 3		MVA ME-TRAP	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Group 1	15.0	3.2 - 37.9	15.0	3.2 - 37.9	10.0	1.2 - 31.7	5.6	0.2 - 27.3	0.0	0.0 - 19.5
Group 2	5.9	0.2 - 28.7			5.9	0.2 - 28.7	0.0	0.0 - 20.6		
Total	10.8	3.0 - 25.4	15.0	3.2 - 37.9	8.1	1.7 - 22.0	2.9	0.1 - 15.3	0.0	0.0 - 19.5

Table 19: Percentage of subjects with at least one report of grade 3 unsolicited AE in the 30 day period post vaccination (Days 0-29)

	RTS,S/AS01B Dose 1		ChAd63 ME-TRAP		RTS,S/AS01B Dose 2		RTS,S/AS01B Dose 3		MVA ME-TRAP	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Group 1	25.0	8.7 - 49.1	30.0	11.9 - 54.3	35.0	15.4 - 59.2	55.6	30.76 - 78.47	35.29	14.21 - 61.67
Group 2	23.5	6.8 - 49.9			35.3	14.2 - 61.7	37.5	15.2 - 64.57		
Total	24.3	11.8 - 41.2	30.0	11.9 - 54.3	35.1	20.2 - 52.5	47.1	29.78 - 64.87	35.29	14.21 - 61.67

Table 20: Percentage of subjects with at least one report of unsolicited AE possibly, probably or definitely related to vaccination in the 30 day period post vaccination (Days 0-29)

3.7.2.28. Adverse Events following ChAd63 ME-TRAP vaccination

The majority of solicited adverse events (AEs) following ChAd63 ME-TRAP vaccination in this study were mild in severity and all resolved. Pain and headache were the most commonly reported AEs in the first 7 days following vaccination with ChAd63 ME-TRAP, each being reported by 55% of subjects. Other commonly reported AEs were feverishness, fatigue and myalgia. Occasional moderate solicited AEs were reported, but there were no reports of severe solicited AEs in the 7 days following vaccination. A graph of solicited adverse events following vaccination with

ChAd63 ME-TRAP can be found in Figure 17. Full tabulation of solicited AEs by group, severity and frequency can be found in the safety appendix.

Unsolicited AEs were processed as outlined above and summary data can be found in Table 18, Table 19, and Table 20. Full listing of all unsolicited AEs with frequency, severity and relationship to vaccination assessments can be found in the supplementary appendix Table 57 to Table 64.

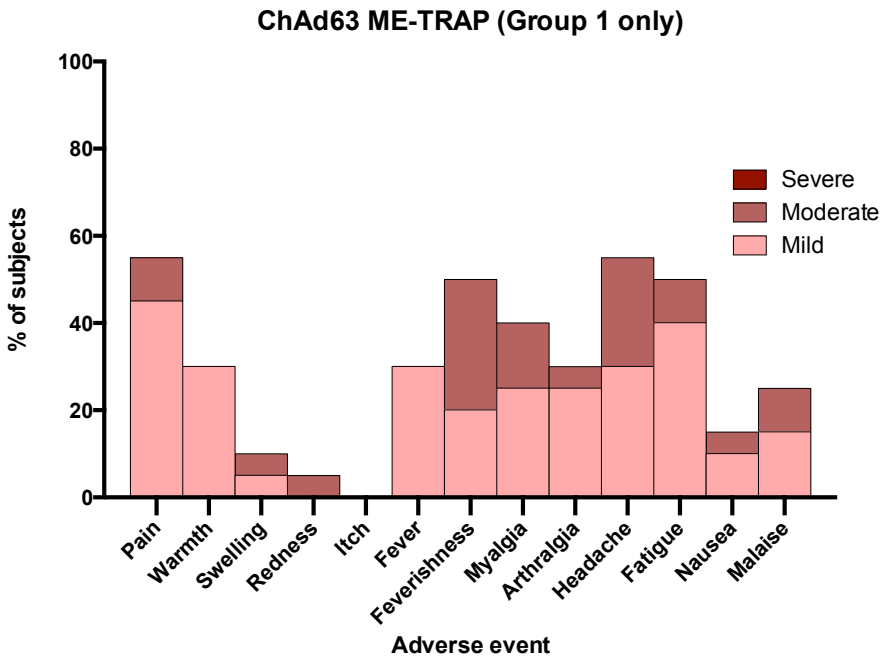


Figure 17: Solicited reactogenicity in the 7 day period following vaccination with ChAd63 ME-TRAP

3.7.2.29. Adverse Events following MVA ME-TRAP vaccination

The majority of solicited adverse events (AEs) following MVA ME-TRAP vaccination in this study were mild in severity and all resolved. Pain was reported by 100% of volunteers in the first 7 days following vaccination, with 5.9% reporting grade 3 pain. Other commonly reported solicited AEs were fatigue (88.2%), feverishness (88.2%) and headache (76.5%). Occasional moderate solicited AEs were reported, and grade 3 (severe) pain, feverishness, myalgia and fatigue were reported in a small number

of subjects. A graph of solicited adverse events following vaccination with MVA ME-TRAP can be found in Figure 18. Full tabulation of solicited AEs by group, severity and frequency can be found in the safety appendix.

Unsolicited AEs were processed as outlined above and summary data can be found in Table 18, Table 19, and Table 20. Full listing of all unsolicited AEs with frequency, severity and relationship to vaccination assessments can be found in the supplementary safety appendix.

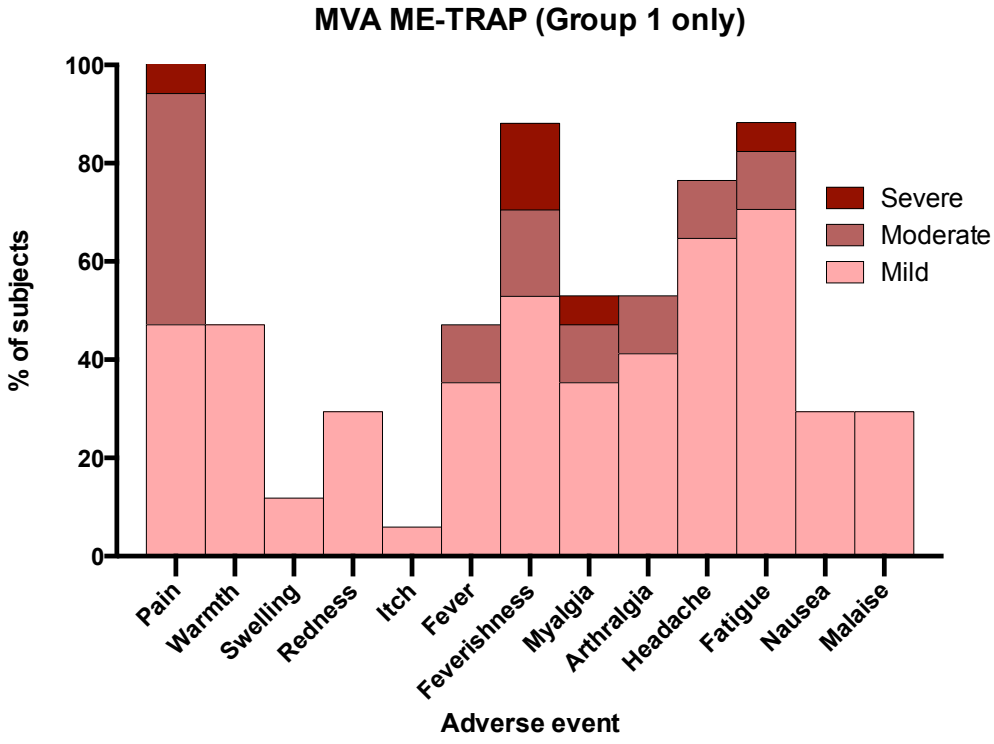


Figure 18: Solicited reactogenicity in the 7 day period following vaccination with MVA ME-TRAP

3.7.3. Immunogenicity

3.7.3.30. Humoral response to vaccination

Anti-CS antibodies (Abs) were measured in all vaccinated subjects at baseline, at key timepoints during the vaccination schedule, and following CHMI (Figure 19). Serum anti-CS Abs peaked at C-1 in both vaccinated groups with peak geometric mean titres (GMT) of 1733 EU [95% CI 1240-2422] and 1824 EU [95% CI 1330-2502] in groups 1 and 2 respectively. Anti-CS Ab GMTs at C-1 were not significantly different between group 1 and group 2 (Mann-Whitney $p>0.99$). (Figure 20) Anti-CS Ab GMTs at C-1 were higher in protected subjects than non-protected subjects, but this did not reach significance, (protected GMT 1985 EU [95% CI 1584-2487]; non-protected GMT 1177 [95% CI 627-2209]; Mann-Whitney $p=0.053$). Anti-CS Ab titre correlated with *P. falciparum* parasite density at day 7.5 (Spearmans r=-0.4, p=0.018). (Figure 21). Avidity of anti-CS Abs was not significantly different at any timepoint when comparing protected subjects with non-protected subjects, however a significant increase in avidity between Day 28 and C-1 was observed in protected subjects, but not non-protected subjects (p=0.001 and p>0.99 respectively, Wilcoxon matched-pairs test). (Figure 22) Avidity also increased between Day 56 and C-1 in protected but not non-protected 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)**.**

Anti-TRAP IgG antibodies (Abs) were measured in Group 1 subjects only (Figure 23) Geometric mean titres (GMTs) peaked on the day before challenge (C-1) at 947 Elisa

Units (EU) [95% CI 617-1455]. There was no boosting of anti-TRAP IgG after CHMI, which is a finding consistent with previous studies of ChAd63/MVA ME-TRAP.[79,80] No association was detected between anti-TRAP IgG levels and efficacy (Spearman's r=-0.25, p=0.3).

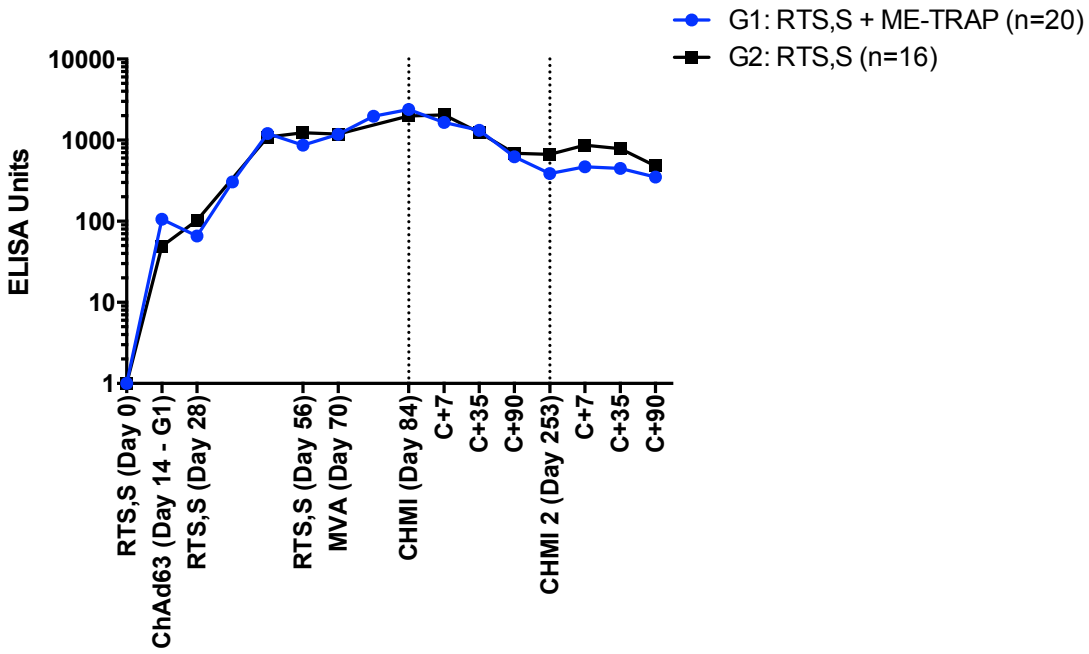


Figure 19: Anti-circumsporozoite protein (CS) immunoglobulin G (IgG) antibody responses after vaccination with RTS,S/AS01B plus ChAd63-MVA ME-TRAP (Group 1; blue) or RTS,S alone (Group 2; black). Line represents group median.

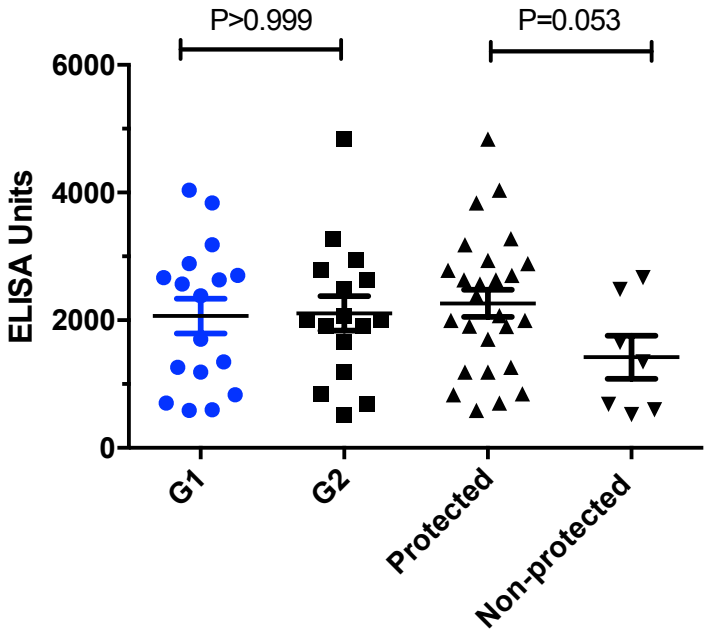


Figure 20: Comparison of Anti-CS IgG antibody responses between Group 1 (Blue) and Group 2 (Black) as measured on the day before CHMI (C-1). No significant difference (NSD) P>0.999 by Mann-Whitney analysis. Comparison of anti-CS IgG antibody responses in volunteers that were sterilely protected or not. Lines represent geometric mean.

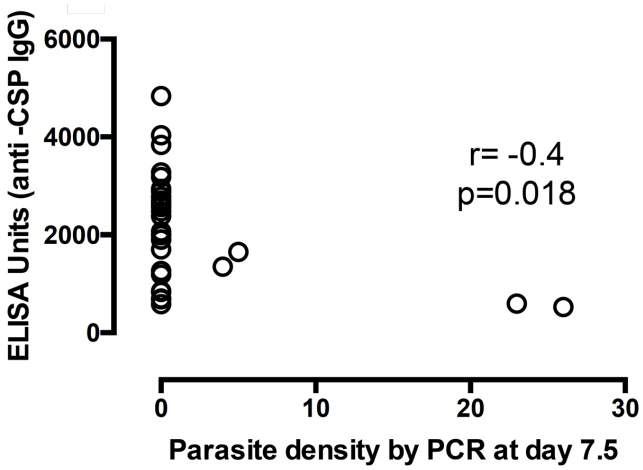


Figure 21: Correlation between anti-CS IgG titres at C-1 and parasite density at day 7 post-challenge. Spearman's r=-0.4, p=0.018.

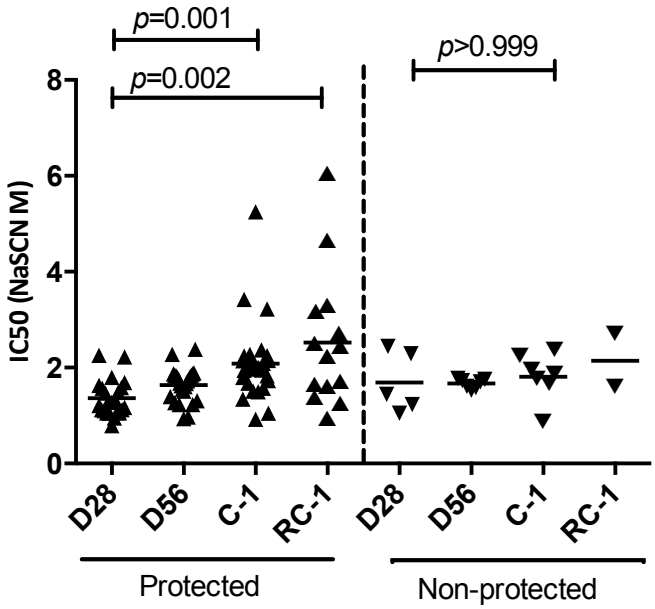


Figure 22: Avidity of total IgG against the NANP repeat region of CSP. Significant increase in avidity between D28 and C-1 in protected, but not non-protected volunteers. P=0.001, Wilcoxon matched-pairs test. Avidity of total IgG remained significantly higher at time of 2nd CHMI (RC-1), compared to D28. P=0.002, Mann-Whitney test. Lines represent geometric mean

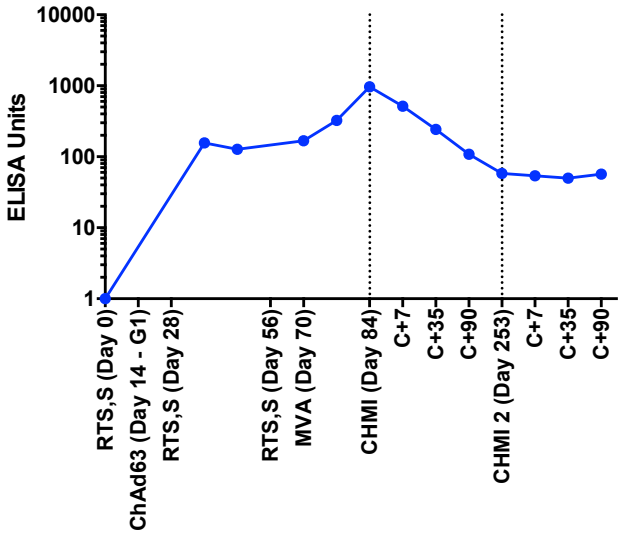


Figure 23: Median anti-thrombospondin adhesion protein (TRAP) immunoglobulin G (IgG) antibody responses after vaccination with RTS,S/AS01B plus ChAd63-MVA ME-TRAP (Group 1 subjects only).

3.7.3.31. Cellular response to vaccination

T-cell responses against ME-TRAP were measured in all Group 1 subjects by ex-vivo interferon-gamma (IFNγ) ELISPOT (

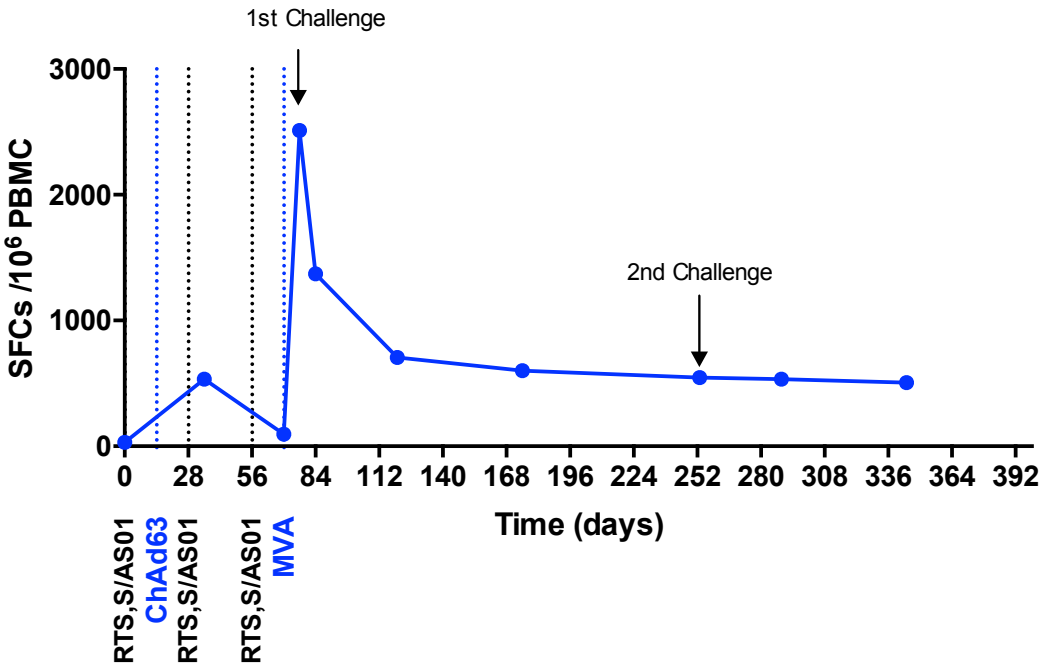


Figure 24). Peak T-cell responses against TRAP after ChAd63 ME-TRAP vaccination were detected 21 days after vaccine administration; GM 539 spot forming cells (SFC) per million peripheral blood mononuclear cells (PBMC) [95% CI 300-968]. Peak responses against TRAP after MVA ME-TRAP vaccination were detected 7 days after vaccine administration; 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 25). Responses peaked in Group 1 at C-1 (4 weeks after final dose of RTS,S/AS01B); median of 75 SFC [IQR 29-159] with responses at 25 SFC [IQR 15-57] 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).

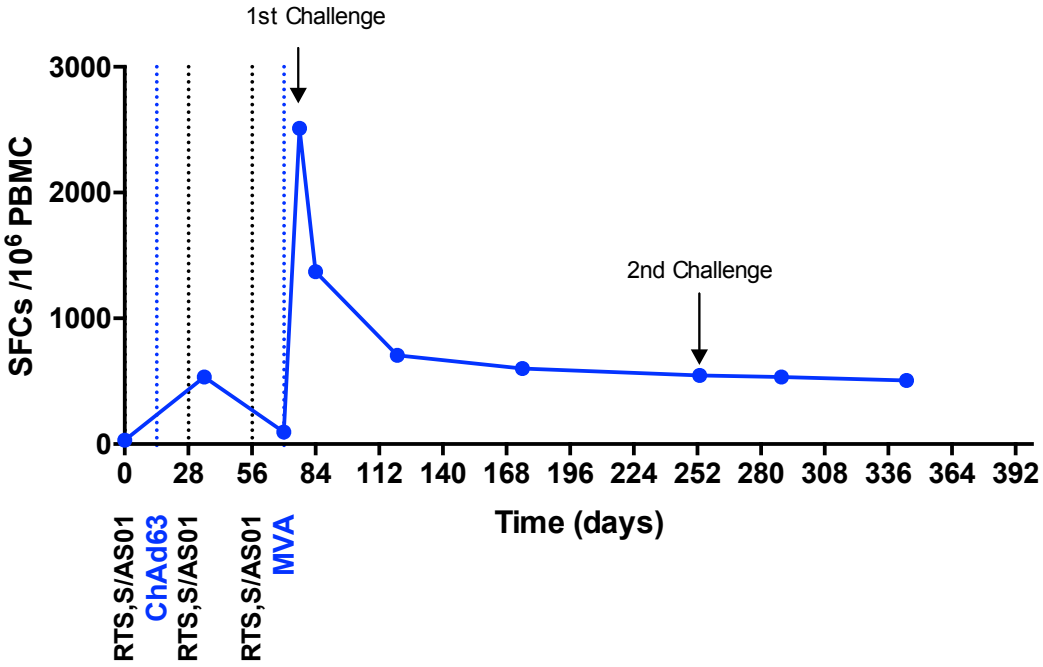


Figure 24: Median antigen-specific T-cell responses to ME-TRAP vaccination measured by IFNγ ELISPOT

C.

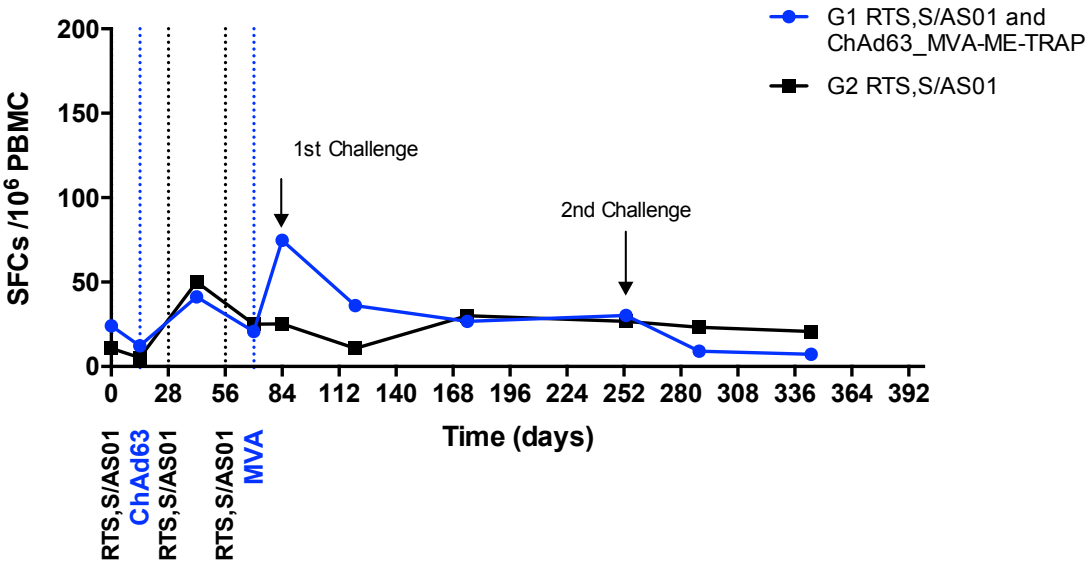


Figure 25: Median antigen-specific T-cell responses to CSP peptide pools are shown for Group 1 (RTS,S/AS01 and ME-TRAP, blue line) and Group 2 (RTS,S/AS01, black line).

Flow cytometry using Intracellular Cytokine Staining (ICS) was performed on cryopreserved PBMC for CS and HBsAg at day 42 after first vaccination and C-1. Responses were described as the number of cells per million $CD4^+$ or $CD8^+$ T cells expressing at least two markers from CD154 (CD40 ligand), IFNγ, IL-2 and TNFα (Figure 26). A peak in CS-specific $CD4^+$ T cell responses was observed at day 42 (two weeks after the second dose of RTS,S/AS01B) in both vaccinated groups. No significant differences were detected between Group 1 and 2 either at either day 42 or C-1 (Figure 27). No significant peaks in CS-specific $CD8^+$ T cell responses were detected (Figure 26). A positive correlation was detected between the number of polyfunctional $CD4^+$ T cells at D42 and serum anti-CS IgG titres at C-1 (Figure 27, 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 28).

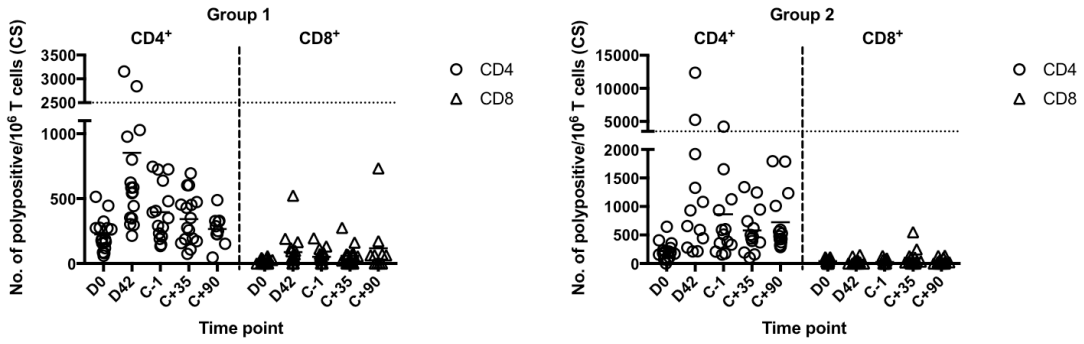


Figure 26: Number of CSP-specific polypositive CD4+ or CD8+ T cells per million in Group 1 (left) and 2 (right) respectively

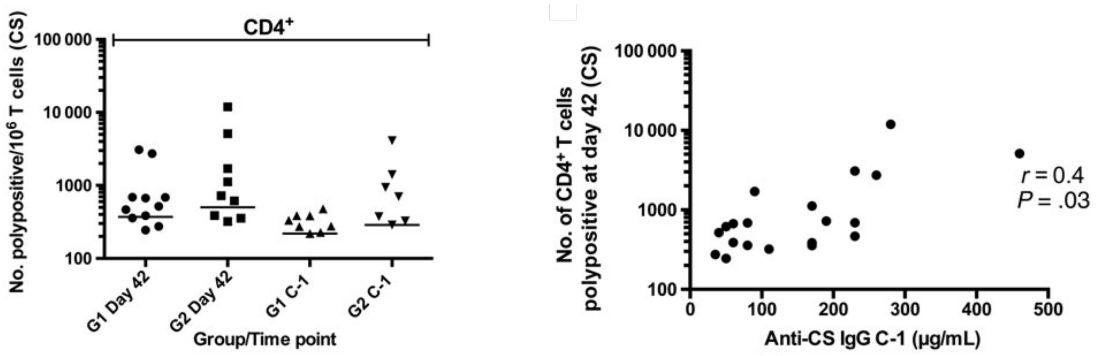


Figure 27: (left) Comparison of CD4+ polypositive T cells at peak time point post vaccination (day 42) and day before CHMI (C-1) for Group 1 (G1) and 2 (G2). (right) Correlation between peak CSP-specific CD4+ polypositive frequency and anti-CS IgG level at C-1 (r=0.4, p=0.03, Spearman's test)

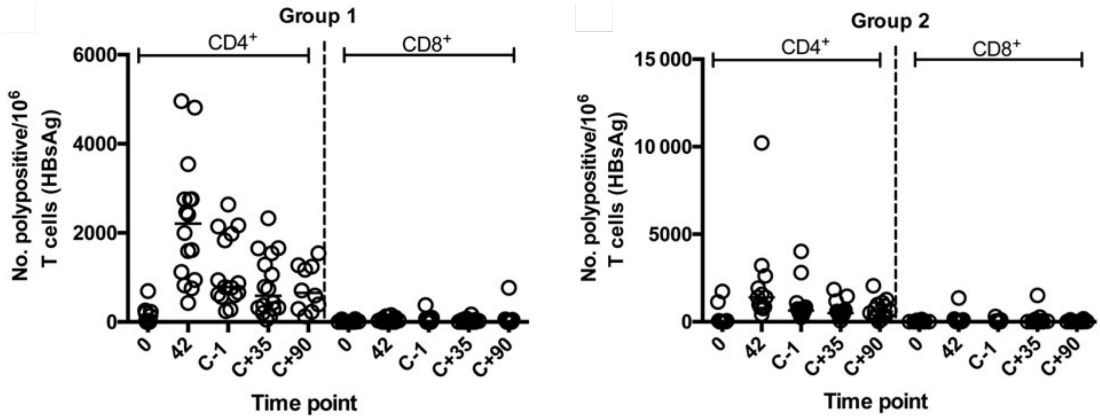


Figure 28: Number of HBS-specific polypositive CD4+ or CD8+ T cells per million in Group 1 (left) and 2 (right) respectively.

Flow cytometry was also performed on freshly isolated PBMCs collected at C-1, using CS peptides on all vaccinated subjects, and using ME-TRAP peptides on Group 1 subjects only. 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 the vaccine insert TRAP strain and the CHMI TRAP strain (Figure 29, Figure 30). Positive

responses to CS were measured in 15/17 (83%) group 1 volunteers compared to 9/16 (56%) volunteers in group 2 (Figure 31). There was also a significantly higher frequency of IFNγ-producing $CD4^+$ T cells in group 1.

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 individual peptide pool, (Figure 32) a significant trend to higher responses in Group 1 compared with Group 2, 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 multi-epitope string of ME-TRAP contains 2 epitopes present in pool 1: CD8 epitope cp26 KPKDELDY; and CD4 epitope DPNANPN, as part of longer ME sequence DPNANPNNVDPNANPNV (Table 21). 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.

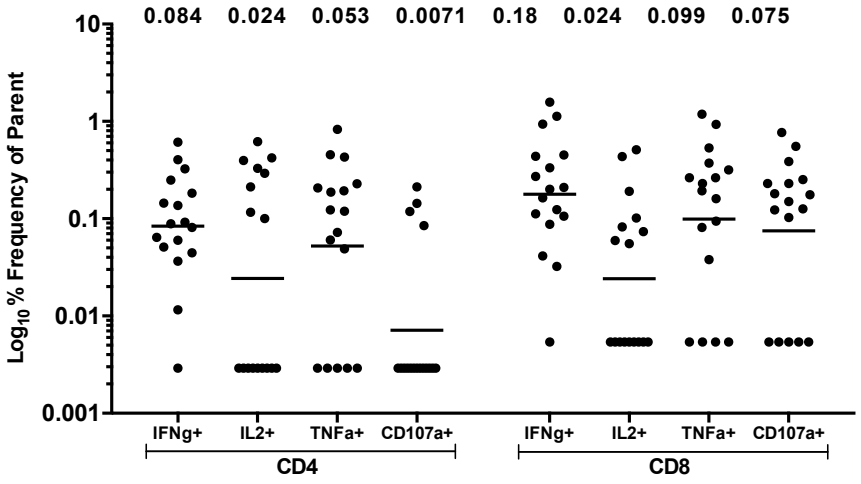


Figure 29: Intracellular cytokine staining of PBMC one day prior to CHMI (27 days post final RTS,S vaccination and 13 days post MVA ME-TRAP vaccination) Geometric mean of each response to stimulation with TRAP T9/96 peptides (homologous to vaccine insert) by Group 1

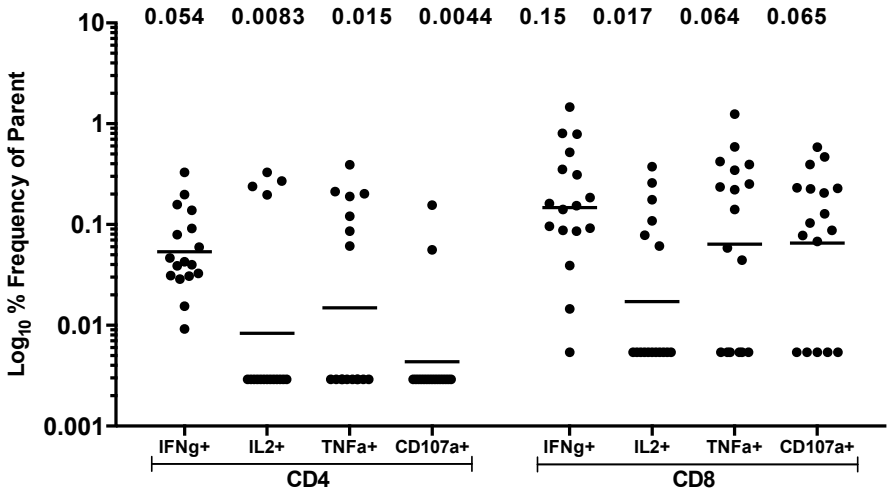


Figure 30: Intracellular cytokine staining of PBMC one day prior to CHMI (27 days post final RTS,S vaccination and 13 days post MVA ME-TRAP vaccination) Geometric mean of each response to stimulation with TRAP 3D7 peptides (homologous to CHMI challenge strain) by Group 1

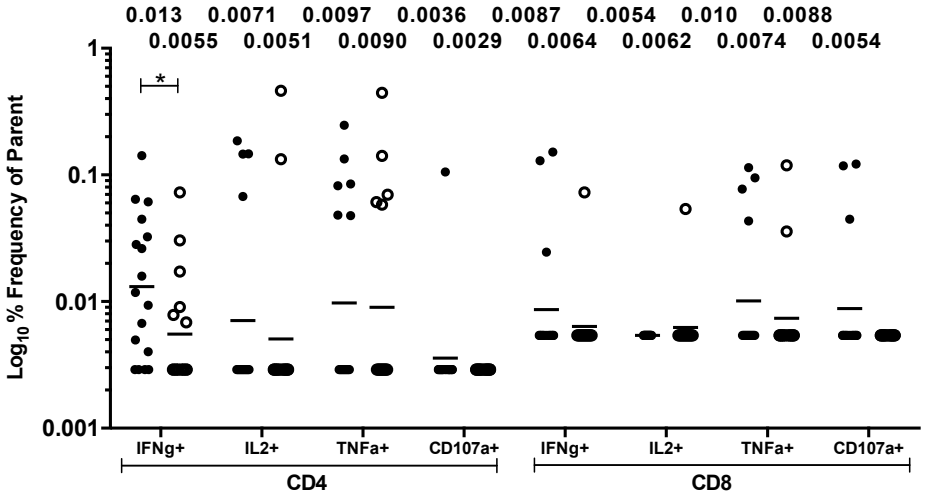


Figure 31: Intracellular cytokine staining of PBMC one day prior to CHMI (27 days post final RTS,S vaccination and 13 days post MVA ME-TRAP vaccination) Geometric mean of each response to stimulation with CS peptides by Group 1 and 2.

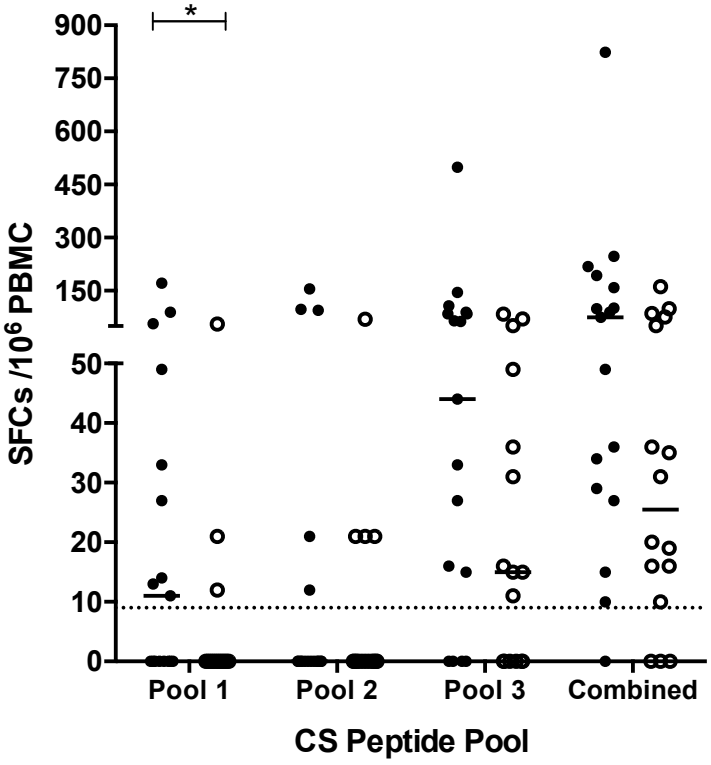


Figure 32: Ex-vivo IFN-γ ELISpot responses of Group 1 and 2 volunteers to CS peptides split into 3 peptide pools and a combined pool, with background subtracted. Dotted line shows the median background ELISpot response, setting the positive response threshold.

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, DNANPN 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

Table 21: Comparison of peptide sequences present in Multi-Epitope string of ME-TRAP and the T cell region of RTS,S (from Lalvani et al [93])

3.8. Discussion

Previous CHMI trials have demonstrated partial efficacy of both RTS,S/AS01B and ChAd63-MVA encoding ME-TRAP [24,25,79,80] but this study is the first in which RTS,S/AS01B and ChAd63-MVA ME-TRAP have been given to subjects in the same vaccine regimen. This study demonstrates that administering these vaccines sequentially within a single vaccine regimen 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/AS01B or ChAd63-MVA ME-TRAP were given alone in a malaria-naïve adult population. [24,25,79,80]

Furthermore, this study shows that these vaccine candidates remain immunogenic when they are combined into a single regimen. 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.[79] Correlation of pre-CHMI anti-CS

antibody titres with protection has previously been demonstrated,[25] however, in this study, geometric mean titres of anti-CS antibodies were not significantly higher in protected subjects at C-1 than non-protected subjects (p=0.053). There was no correlation with protection, however between any specific T cell assays for either TRAP or CS antigens or for TRAP-specific IgG.

In this study, a high level of protective efficacy was observed in both vaccine arms. (Figure 7, Table 13) 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). When subjects who were protected at the initial CHMI were invited back to undergo repeat CHMI, 6 months after initial CHMI, this high level of vaccine efficacy was also seen to be durable with 87.5% and 83.3% of initially protected subjects who underwent repeat CHMI remaining protected in Groups 1 and 2 respectively. (Figure 13)

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 (Figure 8, Figure 9, Table 15). The trends observed in this study for initial challenge, re-challenge 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. Prior to this study, the highest recorded sterile efficacy from a CHMI trial was published in 2013. A CHMI study of the cryopreserved whole sporozoite (PfSPZ) vaccine reported sterile efficacy of 100% in a high dose regimen that consisted of 5 doses of 1.35 x 10^5

parasites.[91] However, the number of vaccinated subjects in this dose group who underwent CHMI was very small (n=6). In addition, only 5/6 (83.3%) of the unvaccinated controls developed blood stage infection, raising concerns over the infectivity of the parasites used in the CHMI, and therefore the adequacy of the infective challenge. At the time of completion of this trial, the results observed, represented the highest reported sterile vaccine efficacy in any CHMI study in which all the control subjects were infected. Since completion of this trial, higher efficacy has been reported, [43] but this will be discussed in greater detail in Chapter 4.

In this trial, a higher level of protective efficacy in the RTS,S/AS01B alone group (75%) was observed than in most prior CHMI studies of this vaccine regimen.[24,25]. The mean time to malaria diagnosis 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.[25] 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 of vaccinated CHMI trial subjects both in the current trial, and for historical comparison. It is also possible, that the efficacy of RTS,S/AS01B against CHMI is actually higher than previously reported, and 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. This 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, as had previously been reported for a 3 dose standard regimen of RTS,S/AS01B.[25] 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 limited, due to the small numbers of subjects and the unexpectedly high efficacy of the RTS,S/AS01B alone regimen. 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/AS01B alone group. A possible alternative approach is to leave a longer period post immunisation to allow antibody and antigen specific T-cell numbers to wane and thereby provide greater power to detect additive or synergist effects of combination vaccines. Challenge studies remain a quick, safe and effective method of rapidly assessing vaccine efficacy in human subjects,[95,97,105] but further consideration of adapting the current model to better increase statistical power is warranted. This may require a specific focus on the practical limitations of CHMI studies with challenge at 3 to 4 weeks after the last vaccination in future trial designs.

In this trial subjects protected at initial CHMI were invited to undergo a re-challenge at 6 months after the initial CHMI. In the re-challenge, 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 6 to 7 months after the immunisations 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/AS01B administered three times[25] 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 the results of phase 3 evaluation of RTS,S/AS01, efficacy was seen to wane to very low levels after the first year following vaccination, especially without a booster dose at 20 months.[28] When considering the potential effectiveness of an experimental vaccine regimen in a real-world setting, a significant limitation of extrapolating from studies that utilise a challenge model is that subjects are often challenged at the peak of vaccine immunogenicity, thereby increasing the likelihood of protection. It is therefore, encouraging in this study to observe that vaccine induced protection of these regimens remains high >6 months after the final dose of vaccine. One argument against re-challenge of subjects protected at initial CHMI is that the *P. falciparum* inoculum used in the CHMI process essentially acts as an immunogenic boost to the vaccine regimen, thereby increasing the likelihood of protection at re-challenge. Possible methods to mitigate this effect would be to use the same controls for comparison in both the initial CHMI and the re-challenge, or to vaccinate a second cohort of each vaccine arm in the trial, who do not take part in the initial CHMI, but instead undergo first challenge at 6 months after last vaccination.

This trial has generated data from a combined vaccine regimen in which subjects received a total of five vaccinations over a 10-week period. A vaccination schedule consisting of five doses is unlikely to be practical for widespread deployment, especially in resource limited areas with poor healthcare infrastructure. The results of this trial are encouraging for further evaluation of a vaccine regimen targeting multiple stages of the malaria parasite life cycle, and further studies should be carried out to evaluate the effect of simplifying such a vaccination schedule. Chapter 4 of this thesis describes a study that evaluated the concomitant administration of RTS,S/AS01B with viral vectors expressing ME-TRAP, thereby reducing the total number of vaccinations in a more practical schedule for potential deployment.

Chapter 4

Safety and Efficacy of Novel Malaria Vaccine Regimens of RTS,S/AS01B alone, or with Concomitantly Administered ChAd-MVA Vectored Vaccines Expressing ME-TRAP (VAC059)

4. Safety and Efficacy of Novel Malaria Vaccine Regimens of RTS,S/AS01B alone, or with Concomitantly Administered ChAd-MVA Vectored Vaccines Expressing ME-TRAP (VAC059)

4.1. Authorship Statement

The work presented in this Chapter is part of a collaborative effort. Unless otherwise stated, all work was undertaken by me.

The study was designed in collaboration with staff at GlaxoSmithkline and PATH MVI. The conduct of the trial was greatly assisted by the Jenner Institute Clinical Trials team (including Alison Lawrie, Rachel Roberts, Ian Poulton, Ruth Payne, Saranya Sridhar, Navin Venkatraman, Oliver Griffiths, Raquel Lopez Ramon) and the teams at Southampton University Hospitals NIHR Wellcome Trust Clinical Research Facility (including Saul Faust), Imperial College London (including Robert Sinden and Graham Cooke), and the Surrey Clinical Research Centre (including David Lewis). This included some assistance with regulatory processes and clinical procedures (volunteer screening, follow up visits etc.).

Nick Edwards performed the qPCR. Katie Ewer, Georgina Bowyer, Carly Bliss and Jonathan Powlson performed the immunological assays, including the ELISAs, ELISPOTs and Flow Cytometry.

Blood samples for FBC, biochemistry and serology (for HIV, Hepatitis B and C) were processed at the Trust laboratories at Oxford University Hospitals' NHS Trust,

Southampton University Hospitals NHS Trust, Imperial College NHS Trust, and Royal Surrey County Hospital NHS Foundation Trust. Malaria films were processed by a team recruited by the Jenner Institute at the Centre for Clinical Vaccinology and Tropical Medicine.

The handling of infectious mosquitoes for CHMI (including transport, preparation for CHMI and dissection) was performed by a team lead by Robert Sinden & Jake Baum at Imperial College London.

Some of the data presented in this chapter is also in the following manuscript that has been submitted to npj Vaccines in October 2017:

- **Rampling T**, Ewer K, Hill AVS *et al*. Safety and Efficacy of Novel Malaria Vaccine Regimens of RTS,S/AS01B alone, or with Concomitant ChAd-MVA Vectored Vaccines Expressing ME-TRAP.

4.2. Introduction

In this phase I/IIa, open-label, CHMI study, I assessed the safety, immunogenicity and efficacy of a vaccine schedule combining two distinct candidate vaccine types in a concomitant immunisation regimen. This expands on the work presented in Chapter 3 using the same two vaccine types (one inducing very high titre antibodies to CS using RTS,S/AS01B and the other inducing potent specific T cell responses to TRAP using viral vectors), but this time evaluating concomitant administration. As discussed in section 1.4.8.4, two separate studies have now demonstrated high efficacy when a fractional 3rd dose of RTS,S/AS01B is delivered on a 0, 1, 7-month

schedule, but it has never been assessed in a 0, 1, 2-month schedule. Therefore, in addition to concomitant administration of vaccines, the effect of a fractional third dose (1/5th standard dose) of RTS,S/AS01B delivered on a 0, 1, 2-month schedule was assessed.

4.3. Objectives

4.3.1. Primary Objective:

To assess the efficacy (occurrence of *P. falciparum* parasitemia, assessed by blood slide) of a combination immunisation regimen with ChAd63/MVA ME-TRAP given concomitantly with two different dosing regimens of RTS,S/AS01B, and of RTS,S/AS01B alone at either full standard dose or with a reduced third dose, against malaria sporozoite challenge, in healthy malaria-naïve volunteers.

To assess the safety of a combination immunisation regimen with ChAd63/MVA ME-TRAP given concomitantly with two different dosing regimens of RTS,S/AS01B, and of RTS,S/AS01B alone at either full standard dose or with a reduced third dose, in healthy malaria-naïve volunteers.

4.3.2. Secondary Objectives:

To assess immunogenicity generated in malaria naïve individuals of two different dosing regimens of RTS,S/AS01B given concomitantly with ChAd63/MVA ME-TRAP, and of RTS,S/AS01B alone at either full standard dose or with a reduced third dose.

To assess the efficacy (measured as time to *P. falciparum* parasitemia assessed by blood slide, by PCR, and parasite density dynamics assessed by PCR) of a combination immunisation regimen with ChAd63/MVA ME-TRAP given concomitantly with two different dosing regimens of RTS,S/AS01B, and of RTS,S/AS01B alone at either full standard dose or with a reduced third dose, against malaria sporozoite challenge, in healthy malaria-naïve volunteers.

4.3.3. Tertiary Objectives:

To assess the long term protective efficacy of two different dosing regimens of RTS,S/AS01B given concomitantly with ChAd63-MVA ME-TRAP and of RTS,S/AS01B alone at either full standard dose or with a reduced third dose by re-challenge of volunteers exhibiting sterile protection.

4.4. Participants

Recruitment and vaccination was conducted at four UK sites, the Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Oxford University Hospitals NHS Trust site, the NIHR Wellcome Trust Clinical Research Facility, University of Southampton, Southampton University Hospitals NHS Trust site, the NIHR Wellcome Trust Imperial College London Clinical Research Facility at the Hammersmith Hospital site, London and the Surrey Clinical Research Centre, University of Surrey. The CHMI procedure was performed as described in Chapter 2 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 infected

mosquitoes supplied by the Department of Entomology, Walter Reed Army Institute of Research, Washington 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). (see Chapter 2: Materials and Methods for the full list of inclusion and exclusion criteria).

4.5. Ethical and Regulatory Approval

Necessary approvals for the study were granted by the UK National Research Ethics Service, Committee South Central – Oxford A (Ref: Ref: 14/SC/0227) and the UK Medicines and Healthcare products Regulatory Agency (Ref: 21584/0333/001-0001). The trial was registered with ClinicalTrials.gov (Ref: NCT02252640). 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.

4.6. Study Design

This Phase IIa, open-label partially randomised challenge trial consisted of 5 cohorts. Initially subjects were allowed to choose whether to be allocated to the vaccination groups or to the control group. Some subjects were ineligible for either Group 3 or 4 due to prior receipt of a viral vectored, non-malaria vaccine, and were therefore

randomised to either Group 1 or 2. All remaining vaccine subjects were randomised to Groups 1-4 (target n=13 per group) to receive 3 sets of vaccinations at 4-week intervals. Group 1 received 3 standard doses (50 μg) of RTS,S/AS01B (R-R-R); Group 2 received 2 standard doses of RTS,S/AS01B followed by a third fractional dose of RTS,S/AS01B at $1/5^{th}$ of the standard dose (R-R-r); Group 3 received 3 standard doses of RTS,S/AS01B in addition to ChAd63 ME-TRAP 5 x 10^{10} virus particles (vp) at week 0 and MVA ME-TRAP 2 x 10^{8} plaque forming units (pfu) at week 4 and week 8 (RA-RM-RM); Group 4 received 2 standard doses of RTS,S/AS01B followed by a third fractional dose of RTS,S/AS01B at $1/5^{th}$ of the standard dose in addition to ChAd63 ME-TRAP 5 x 10^{10} vp at week 0 and MVA ME-TRAP 2 x 10^{8} pfu at week 4 and week 8 (RA-RM-rM). Group 5 (n=4) received no vaccinations.

Week	Group 1 (n=13) R-R-R	Group 2 (n=13) R-R-r	Group 3 (n=13) RA-RM-RM	Group 4 (n=13) RA-RM-rM	Group 5 (n=4) Control	Group 6 (n=4) Control
0	RTS,S/AS01$_B$ (Standard dose)	RTS,S/AS01B (Standard dose)	RTS,S/AS01B (Standard dose) & ChAd63 ME-TRAP	RTS,S/AS01B (Standard dose) & ChAd63 ME-TRAP		
4	RTS,S/AS01B (Standard dose)	RTS,S/AS01B (Standard dose)	RTS,S/AS01B (Standard dose) & MVA ME-TRAP	RTS,S/AS01B (Standard dose) & MVA ME-TRAP		
8	RTS,S/AS01B (Standard dose)	RTS,S/AS01B (1/5 Standard dose)	RTS,S/AS01B (Standard dose) & MVA ME-TRAP	RTS,S/AS01B (1/5 Standard dose) & MVA ME-TRAP		
11	CHMI (n=12)	CHMI (n=12)	CHMI (n=12)	CHMI (n=12)	CHMI	
31-39	Repeat CHMI of sterilely protected volunteers	Repeat CHMI of sterilely protected volunteers	Repeat CHMI of sterilely protected volunteers	Repeat CHMI of sterilely protected volunteers		CHMI

Table 22: VAC059 Clinical Trial Design

All vaccinations were administered intramuscularly (IM) into the deltoid region of the arm. For subjects in Groups 3 and 4, RTS,S/AS01B was administered first followed by viral vector vaccination in the same site no longer than 5 minutes after the RTS,S vaccination. All subjects underwent CHMI by mosquito bite at the same time (3 weeks after third 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.[80] Vaccinated subjects who had not developed blood stage malaria by day 23 after CHMI were deemed to exhibit sterile protection. 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 Chapter 2: Materials and Methods.

4.7. Results

4.7.1. Participants

Recruitment took place between August 2014 and March 2015. Forty five healthy adult volunteers were enrolled. Demographics for the subjects enrolled are shown in Table 12. Seventy-four subjects were screened for eligibility and 45 subjects were identified as eligible. Ten subjects were allocated to each of Groups 1, 2 and 3 to receive R-R-R, R-R-r or RA-RM-RM respectively. Eleven subjects were allocated to Group 4 to receive RA-RM-rM and 4 subjects were allocated to Group 5 to act as unvaccinated infectivity controls. Two subjects were deemed eligible as control subjects but only after Group 5 enrolment was complete. They were kept as backup

subjects in case of last minute withdrawals from Group 3, but never underwent CHMI. Subjects were randomised to group by the study statistician. Vaccinations took place between 5th January 2015 and 27th February 2015. Prior to CHMI, 2 subjects withdrew from group 1, 1 subject withdrew from Group 2, and 2 subjects withdrew from Group 4. There were no withdrawals due to safety concerns and no pre-defined study stopping or holding rules were activated. CHMI was performed on the 23rd and 24th of March 2015. A flow diagram of recruitment can be found in Figure 33.

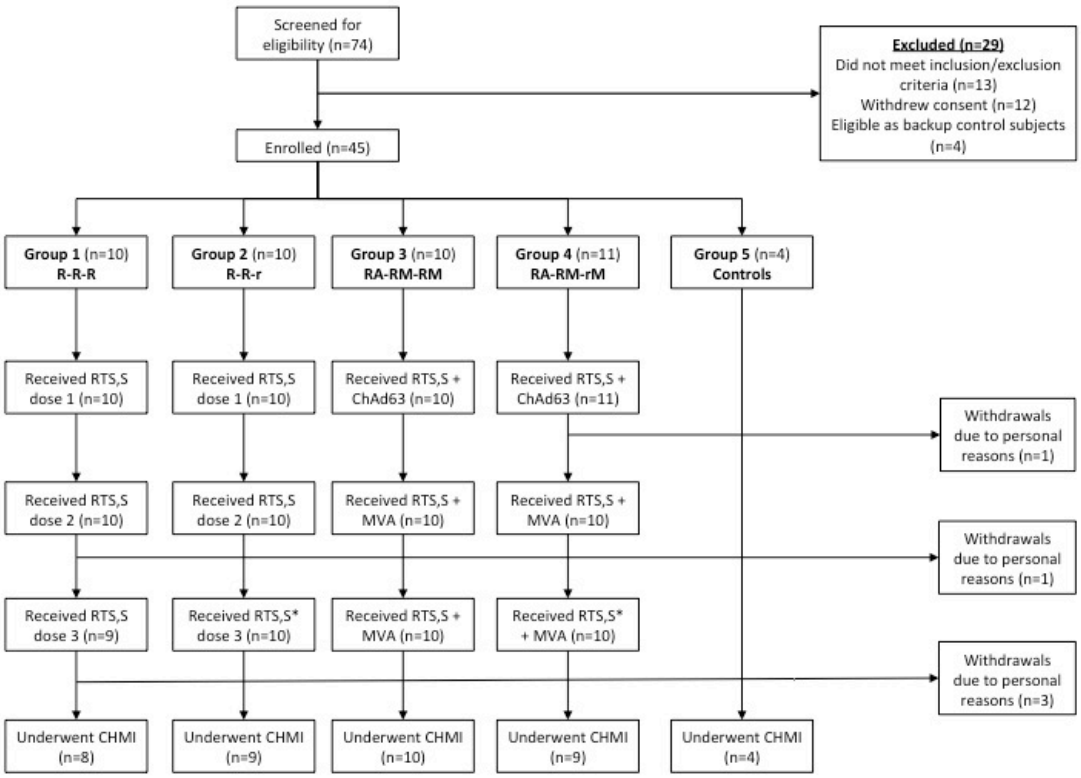


Figure 33: VAC059 flow chart of study design and volunteer recruitment. CHMI = controlled human malaria infection. * Denotes RTS,S/AS01B delivered at 1/5th of the standard dose

	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	Group 5 n (%)	All Groups n (%)
Gender						
Male	6 (60)	7 (70)	3 (30)	6 (54.5)	3 (75)	25 (55.6)
Female	4 (40)	3 (30)	7 (70)	5 (45.5)	1 (25)	20 (44.4)
Country of Birth						
UK	6 (60)	4 (40)	9 (90)	7 (63.6)	4 (100)	30 (66.7)
Other	4 (40)	6 (60)	1 (10)	4 (36.4)	0	15 (33.3)
Age						
Range						
18-20	1 (10)	0	0	0	0	1 (2.2)
21-30	4 (40)	9 (90)	6 (60)	9 (81.8)	4 (100)	32 (71.1)
31-40	5 (50)	0	2 (20)	1 (9.1)	0	8 (17.8)
>41	0	1 (10)	2 (20)	1 (9.1)	0	4 (8.9)
Mean (+/- SD)	29.6 ±5.4	27.5 ±6.2	31.3 ±8.5	28.6 ±6.8	22.4 ±1.0	28.6 ±6.7
BMI						
<18.5	0	0	0	1 (9.1)	0*	1 (2.3)*
18.5-24.9	9 (90)	5 (50)	5 (50)	4 (36.4)	3 (100)*	26 (59.1)*
25-29.9	0	1 (10)	3 (30)	5 (45.5)	0*	9 (20.5)*
>30	1 (10)	4 (40)	2 (20)	1 (9.1)	0*	8 (18.2)*
Mean (+/- SD)	23.4 ±2.9	26.7 ±5.2	26.1 ±5.4	26.0 ±5.9	22.0 ±1.9*	28.6 ±4.9*

Table 23: Demographics of all subjects enrolled in VAC059. *Accurate weight was not recorded for a single subject in Group 5 at screening. Therefore these results have been adjusted accordingly.

4.7.2. Protective efficacy against CHMI

Efficacy was assessed by Controlled Human Malaria Infection (CHMI) with *P. falciparum* by mosquito bite. A total of 40 subjects were challenged over the 2 days and completed subsequent follow up (8 subjects from Group 1, 9 subjects from Group 2, 10 subjects from Group 3, 9 subjects from Group 4 and 4 subjects in Group 5).

4.7.2.32. Sterile efficacy

By day 23 following CHMI, 6/8 subjects in Group 1 remained protected (Vaccine Efficacy (VE) 75%, 95% CI [31.5-93.1]); in Group 2, 8/9 subjects remained protected (VE 88.9%, 95% CI [43.3-98.4]); in Group 3, 6/10 subjects remained protected (VE 60%, 95% CI [25.3-82.7]); in Group 4, 5/9 subjects remained protected (VE 55.6%,

95% CI [20.4-80.5]). All 4 unvaccinated controls in Group 5 were diagnosed with malaria. Pooling the outcome of subjects in Groups 1 and 2, who had received RTS.S/AS01B alone, 14/17 subjects remain protected (82.4%, 95% CI [54.7-93.9]) compared with 11/19 subjects who had received RTS,S/AS01B with viral vectors in Groups 3 and 4 (VE 57.9% 95% CI[33.2-76.3]; p=0.074). Median time to diagnosis was 11.5 days (range 11.5-12, SD=0.22 days) in Group 5, while median time to diagnosis was 15.5, 16.5, 14 and 14.25 days in Groups 1, 2, 3 and 4 respectively.

	Proportion protected (% [95%CI])		**Proportion protected (% [95%CI])**
Group 1	6/8 (**75%** [31.5-93.1]	**Group 1&2**	14/17 (**82.4%** [54.7-93.9]
Group 2	8/9 (**88.9%** [43.3-98.4]		
Group 3	6/10 (**60%** [25.3-82.7]	**Group 3&4**	11/19 (**57.9%** [33.2-76.3]
Group 4	5/9 (**55.6%** [20.4-80.5]		
Group 5	0/6 (**0%**)		

Table 24: Proportion of subjects completely protected by day 23 after CHMI in individual groups and in the pooled RTS,S/AS01B only groups (Groups 1 & 2) and in the RTS,S/AS01B plus viral vectors groups (Groups 3 & 4). Results are shown as a percentage with 95% confidence intervals (95%CI)

All four vaccine regimens demonstrated significantly reduced risk of malaria infection over controls by logrank test (Group 1 *p*=0.0003; Group 2 *p*=0.0002, Group 3 *p*=<0.0001; Group 4 *p*=0.0002). but there was no significant difference in efficacy between any individual vaccine regimen or in pooled comparison of Groups 1 & 2 vs group 3 & 4 (Table 25).

4.7.2.33. Secondary efficacy endpoints

Figure 34 shows the time to malaria diagnosis for the 4 individual vaccine groups (Groups 1, 2, 3 and 4) compared to controls (Group 5). Figure 35 shows the time to malaria diagnosis for the pooled RTS,S/AS01B only groups (Group 1 & 2) and the pooled RTS,S/AS01B plus viral vectors groups (Groups 3 & 4). Figure 36 shows the time to PCR positivity (>20 parasites/mL) and Figure 37 shows the time to 500 parasites/mL by PCR. Mean time to each of these endpoints plus proportion of subjects reaching them is summarized in Table 26, and statistical comparison of time to each endpoint is shown in Table 25. By logrank test, there was a significant effect of each vaccine regimen over controls, in time to either 20 parasites/ml and 500 parasites/ml. There was no difference between any of the individual vaccine regimens, or the pooled comparison of Group 1&2 vs Group 3&4 at reaching either endpoint.

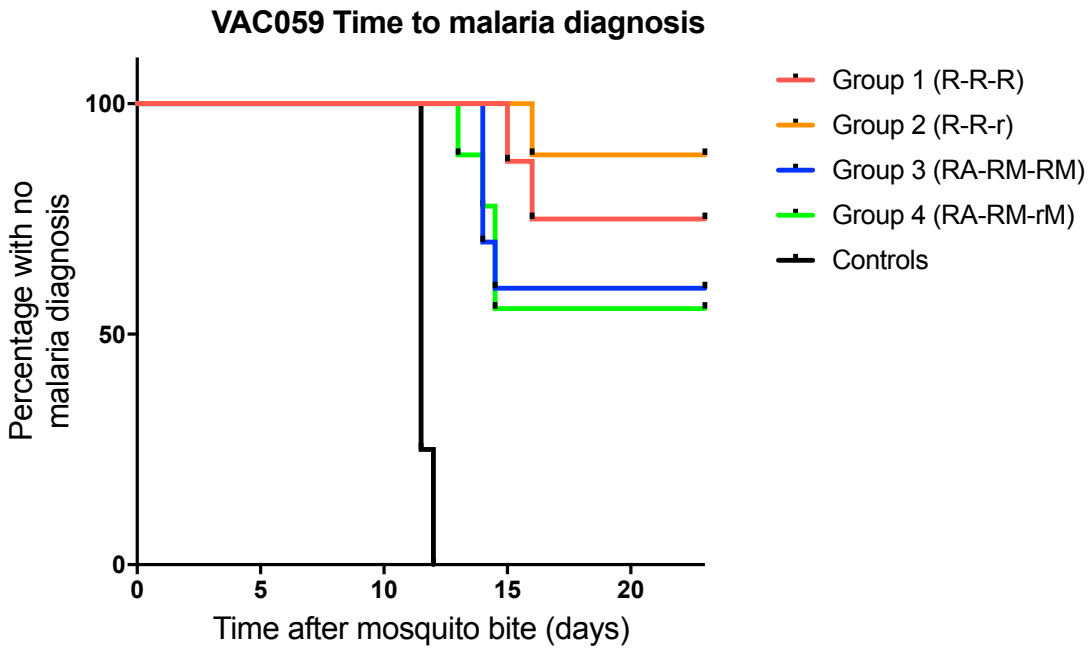


Figure 34: Kaplan-Meier survival analyses of time to malaria diagnosis (primary endpoint) by blood film and/or PCR parasitaemia >500 parasites/ml +/- symptoms in days for the individual vaccine groups and the control group (n=4): Group 1 (R-R-R, n=8), Group 2 (R-R-r, n=9), Group 3 (RA-RM-RM, n=10), Group 4 (RA-RM-rM, n=9)

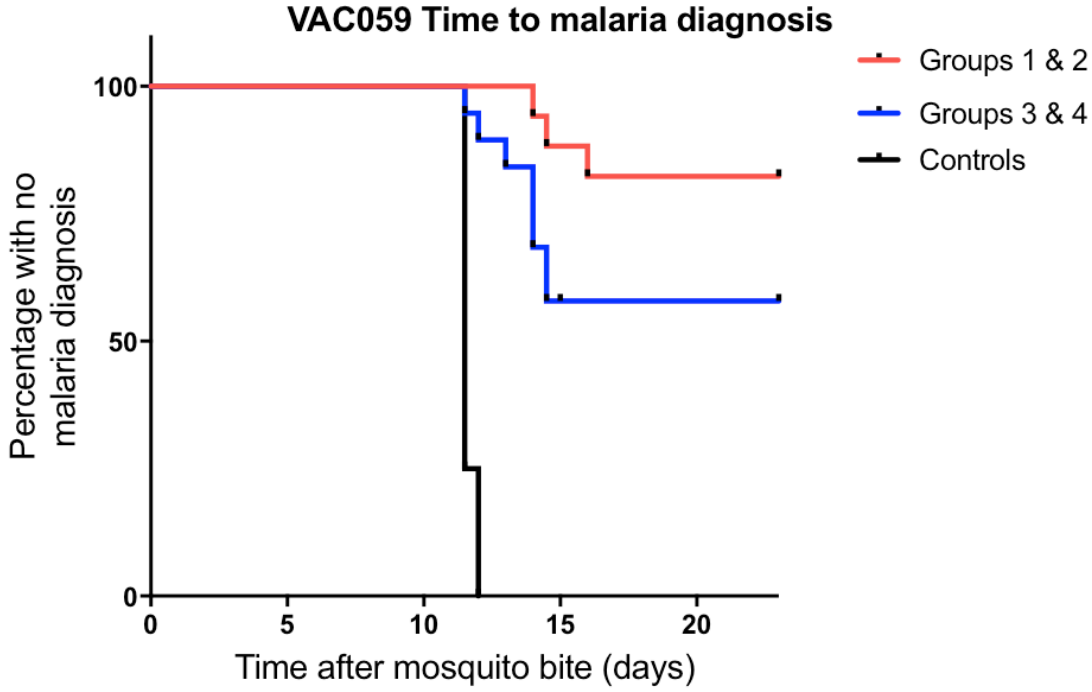


Figure 35: Kaplan-Meier survival analyses of time to malaria diagnosis (primary endpoint) by blood film and/or PCR parasitaemia >500 parasites/ml +/- symptoms in days for the pooled vaccine groups and the control group (n=4): Group 1 & 2 (n=17), Group 3 &4 (n=19)

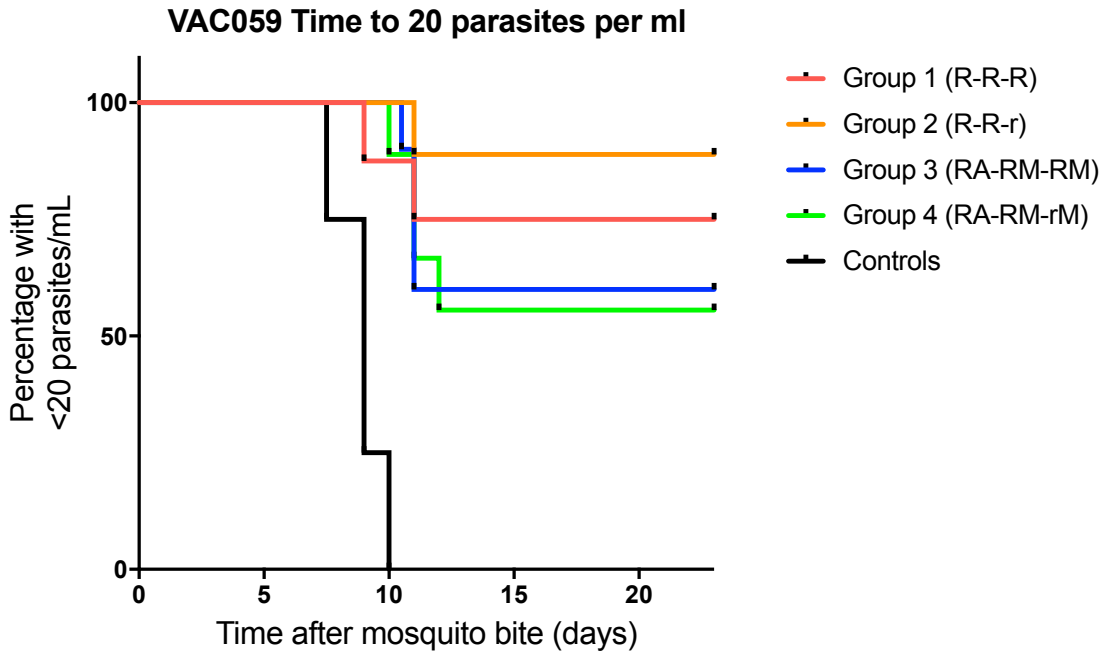


Figure 36: Kaplan-Meier survival analyses of time to 20 parasites/ml (secondary endpoint) by qPCR in days for the individual vaccine groups and the control group.

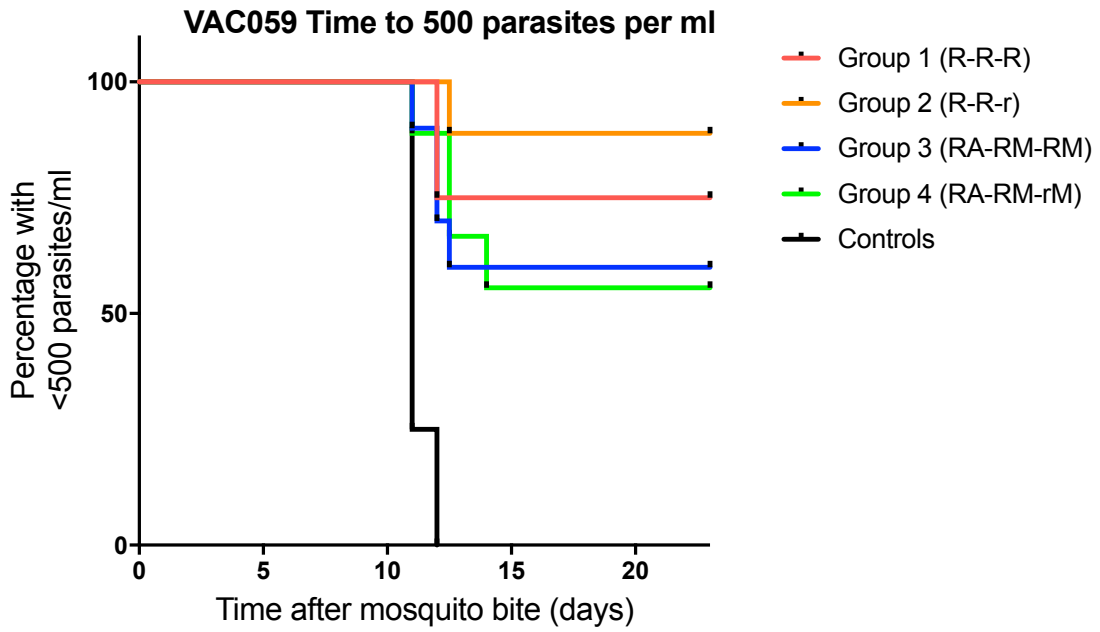


Figure 37: Kaplan-Meier survival analyses of time to 500 parasites/ml (secondary endpoint) by qPCR in for the individual vaccine groups and the control group.

	Fishers Exact Test	Malaria diagnosis		Time to 20p/ml		Time to 500p/ml	
	P vs NP *p*	Logrank *p*	HR [CI]	Logrank *p*	HR [CI]	Logrank *p*	HR [CI]
Gp1 vs controls	0.0606	**0.0003**	**0.1136 [0.0145-0.8915**	**0.0024**	**0.1351 [0.0191-0.9547]**	**0.0023**	**0.1429 [0.0209-0.979]**
Gp2 vs controls	**0.0070**	**0.0002**	**0.0643 [0.0073-0.5648]**	**0.0001**	**0.0555 [0.0057-0.5389]**	**0.0002**	**0.0643 [0.0073-0.5648]**
Gp3 vs controls	0.0849	**<0.0001**	0.1344 [0.0158-1.147]	**< 0.0001**	0.1170 [0.0122-1.125]	**0.0052**	0.2200 [0.0363-1.334]
Gp4 vs controls	0.1049	**0.0002**	0.1466 [0.0184-1.168]	**0.0004**	0.1441 [0.0179-1.163]	**0.0020**	0.2015 [0.0315-1.288]
Gp1 vs Gp2	0.5765	0.4369	2.459 [0.2527-23.93]	0.4369	2.459 [0.2527-23.93]	0.4334	2.474 [0.254-24.09]
Gp1 vs Gp3	0.6380	0.4369	0.5079 [0.1025-2.516]	0.5916	0.6511 [0.1296-3.271]	0.4902	0.5759 [0.1158-2.865]
Gp3 vs Gp4	1.0000	0.8487	0.8830 [0.2202-3.54]	0.8598	0.8917 [0.2225-3.574]	0.4902	0.9943 [0.2487-3.976]
Gp2 vs Gp4	0.2941	0.0992	0.1969 [0.0337-1.151]	0.1215	0.2200 [0.0380-1.274]	0.1215	0.2200 [0.0380-1.274]
Gp1&2 vs controls	**0.0058**	**<0.0001**	**0.0738 [0.0055-0.9877]**	**<0.0001**	**0.0832 [0.0070-0.9846]**	**<0.0001**	**0.0884 [0.0079-0.9863]**
Gp3&4 vs controls	0.0932	**<0.0001**	0.1001 [0.0070-1.425]	**<0.0001**	0.09546 [0.0063-1.44]	**0.0005**	0.1890 [0.0252-1.417]
Gp1&2 vs Gp3&4	0.1560	0.0744	0.3298 [0.1009-1.078]	0.1334	0.3986 [0.1222-1.3]	0.1191	0.3773 [0.1157-1.23]

Table 25: Statistical analysis of the Primary and Secondary efficacy endpoints. Fisher's exact test has been performed to compare absolute numbers of protected versus non-protected subjects within groups. Logrank survival analysis has been performed on each of the 3 secondary efficacy endpoints p values and hazard ratios with 95% confidence intervals on the per protocol cohorts for each of the 3 secondary efficacy endpoints.

Endpoint	Control mean (SD)	Proportion of subjects with delay to endpoint (% [95% CI])					
		Group 1	Group 2	Group 3	Group 4	Group 1 & 2	Group 3 & 4
Time to malaria diagnosis (days)	11.6 ±0.22	8/8 (100)	9/9 (100)	10/10 (100)	9/9 (100)	17/17 (100)	19/19 (100)
Time to 20 parasites/ml (days)	8.9 ±0.89	7/8 (87.5% [52.9-99.4])	9/9 (100% [70.1-100])	9/10 (90% [59.6-99.5])	8/9 (88.9% [56.5-99.4])	16/17 (94.1% [73.0-99.7])	17/19 (89.5% [68.6-98.1])
Time to 500 parasites/ml (days)	11.3 ±0.43	6/8 (75% [40.9-95.6])	9/9 (100% [70.1-100])	7/10 (70% [39.7-89.2])	8/9 (88.9% [56.5-99.4])	15/17 (88.2% [65.7-97.9])	15/19 (78.9% [56.7-91.5]}

Table 26: Secondary efficacy endpoints. Data shown is the proportion of subjects who were delayed in reaching each endpoint. A delay to endpoint is defined here as any subject who reaches the endpoint after the control (Group 5) mean + 2x the standard deviation (SD).

4.7.2.34. Tertiary efficacy endpoints

Due to lower than expected level of protective efficacy in Groups 3 and 4, re-challenge of protected volunteers was not performed. The numbers of protected subjects were too low to reliably produce results of scientific significance.

4.7.3. Safety

4.7.3.35. Serious Adverse Events (SAEs)

In this trial, there have been 2 SAEs and no SUSARs. Neither SAE was deemed related to vaccination, and each is described in detail below. No pregnancies or deaths have occurred.

SAE #1

On the evening of the 14th August 2015 this subject fell from her bicycle and sustained a head injury. She was admitted to the John Radcliffe Hospital in Oxford with concussion and facial injuries. She did not recall the events of the fall from her bicycle. A CT head scan was performed, but no evidence of intracranial bleeding or bony injury was found. She was discharged from hospital on the 15th August 2015. There was no long-term injury or disability sustained as a result of this SAE.

SAE #2

This subject had a cycling accident on the 22nd February 2015, following which she attended Accident and Emergency. She was diagnosed with a fractured right clavicle, and was sent home from hospital with an appointment to return for surgery. She

had an operation on her clavicle on the 25th February under general anaesthesia, and was kept in hospital overnight. She was discharged with a sling and was required to keep it immobilised for 1 month.

4.7.3.36. Solicited Adverse events following vaccination

The majority of solicited adverse events (AEs) following each vaccination in this study were mild in severity and all resolved. Commonly reported adverse events were vaccine site pain, feverishness, myalgia, arthralgia, fatigue, headache and malaise (Figure 38, Figure 39 and Figure 40). Full tabulation of solicited AEs by group, severity and frequency can be found in the safety appendix. A higher frequency of solicited adverse events was reported after the second vaccination, compared with the first or third vaccinations. Of note, 100% of subjects in Groups 3&4 reported local pain following this vaccination, compared with 75% in Groups 1&2. Feverishness was reported in 95% of subjects in Groups 3&4, although only 35% had a measurable fever. A higher frequency of solicited AEs was observed in subjects who received the viral vectors in addition to RTS,S/AS01B (Groups 3&4), but the frequency was only significantly higher when comparing rates of fever, arthralgia and malaise following the first vaccination, swelling following the second vaccination and redness following the third vaccination (Table 27). There was no significant difference in the frequency of severe AEs when comparing Groups 1&2 against Groups 3&4.

A lower frequency of solicited adverse events was observed after the third vaccination in the groups that received a reduced third dose of RTS,S/AS01B (Groups 2&4), although this discrepancy was most pronounced when comparing rates of

feverishness in Group 1 (55.4% reporting any feverishness) against rates in Group 2 (0%, *p*=0.0108).

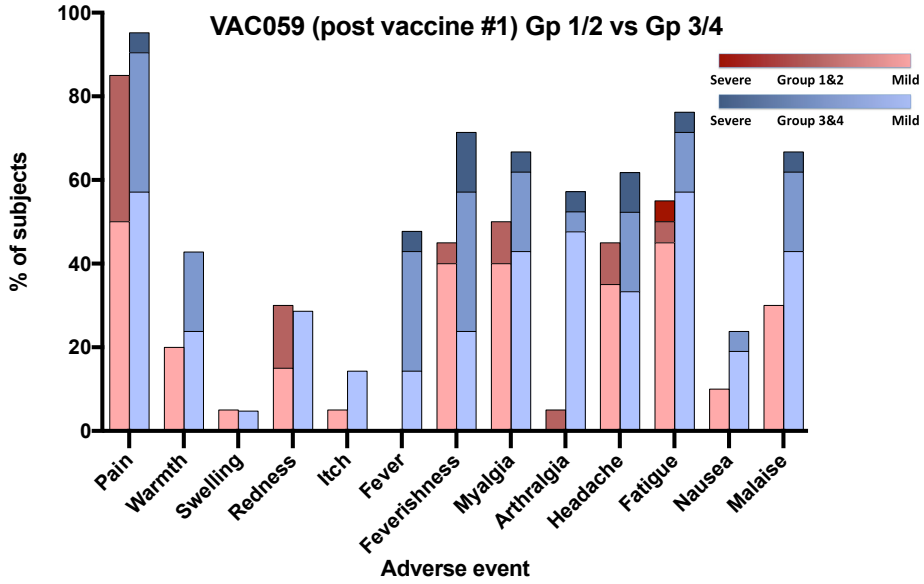


Figure 38: Solicited reactogenicity in the 7-day period following the first vaccination in the trial. Adverse events recorded for subjects in Groups 1 and 2, who received RTS,S/AS01B only, are shown in the red columns. Adverse events recorded for subjects in Groups 3 and 4, who received RTS,S/AS01B and ChAd63 ME-TRAP, are shown in the blue columns. Increasing AE severity (mild, moderate or severe) is represented by darker shading. Each AE is recorded once at maximum severity for each subject.

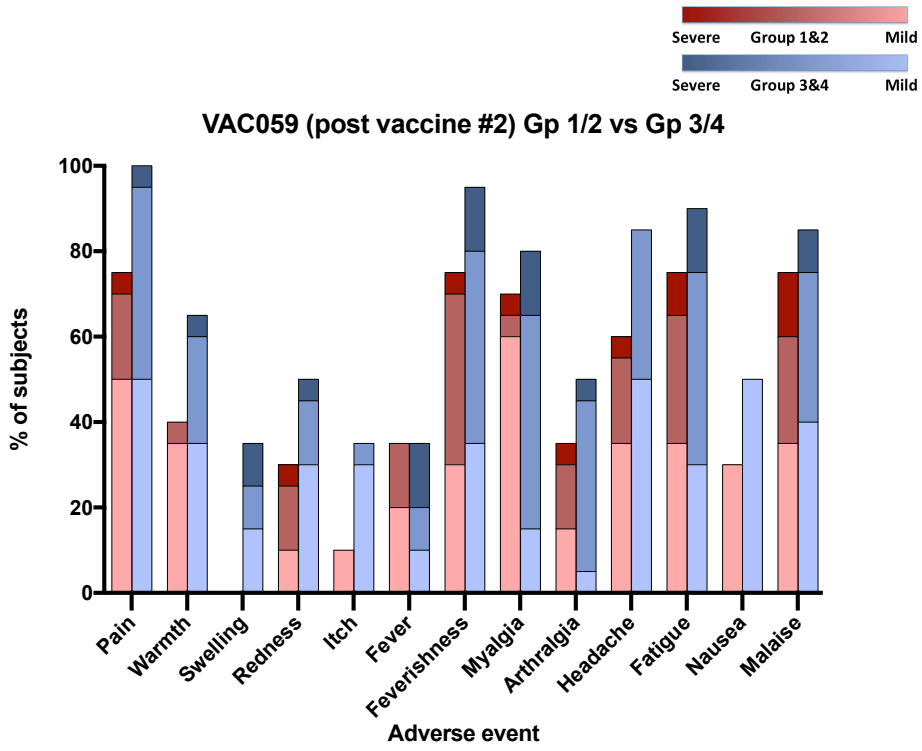


Figure 39: Solicited reactogenicity in the 7-day period following the second vaccination in the trial.

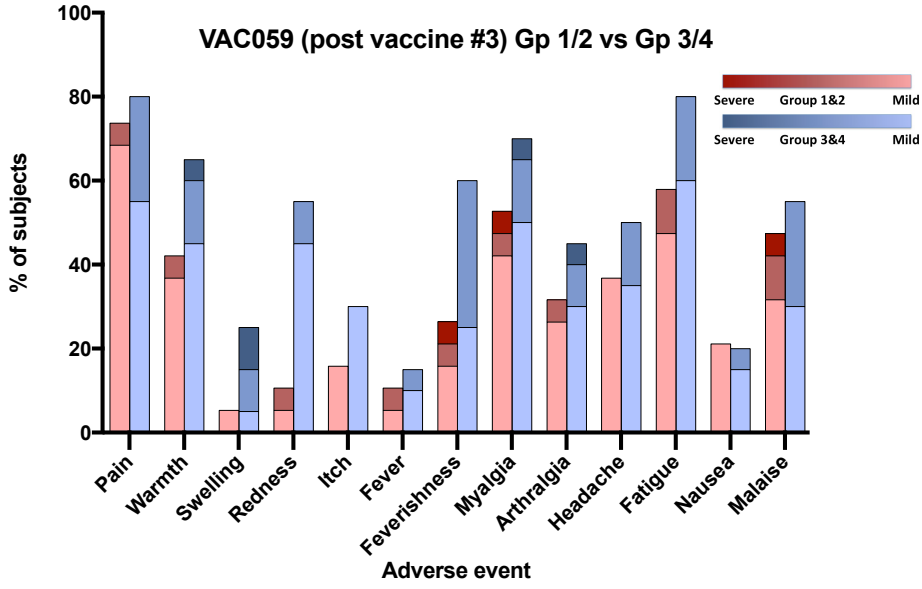


Figure 40: Solicited reactogenicity in the 7-day period following the third vaccination in the trial.

		Vaccine 1	Vaccine 2	Vaccine 3		
		Group 1&2 vs Group 3&4	Group 1&2 vs Group 3&4	Group 1 vs Group 2	Group 3 vs Group 4	Group 1&2 vs Group 3&4
		p	*p*	*p*	*p*	*p*
Pain	**Any**	0.2317	0.731	0.3034	0.582	0.7164
	Severe	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Warmth	**Any**	0.1971	0.2049	0.3698	0.3498	0.2049
	Severe	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Swelling	**Any**	>0.9999	0.0083	>0.9999	>0.9999	0.1818
	Severe	>0.9999	0.4872	>0.9999	>0.9999	0.4872
Redness	**Any**	>0.9999	0.3332	0.2105	>0.9999	0.0057
	Severe	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Itch	**Any**	0.606	0.1274	0.0867	0.1409	0.4506
	Severe	>0.9999	0.1274	>0.9999	>0.9999	>0.9999
Fever	**Any**	0.0005	0.2553	0.2105	>0.9999	>0.9999
	Severe	>0.9999	0.2308	>0.9999	>0.9999	>0.9999
Feverishness	**Any**	0.0578	0.1818	0.0108	0.6499	0.0536
	Severe	0.2317	0.605	0.4737	>0.9999	0.4872
Myalgia	**Any**	0.5303	0.7164	0.3698	0.1409	0.3332
	Severe	>0.9999	0.605	0.3698	>0.9999	>0.9999
Arthralgia	**Any**	0.0005	0.5231	0.3498	>0.9999	0.5145
	Severe	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Headache	**Any**	0.2146	0.1552	0.6499	0.6563	0.5231
	Severe	0.4878	>0.9999	>0.9999	>0.9999	>0.9999
Fatigue	**Any**	0.3264	0.4075	0.6499	0.582	0.176
	Severe	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Nausea	**Any**	0.2379	0.3332	>0.9999	0.582	>0.9999
	Severe	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
Malaise	**Any**	0.0126	0.6948	0.1789	0.3698	0.7524
	Severe	>0.9999	>0.9999	0.4737	>0.9999	0.4872
Any Solicited AE	**Any**	0.4878	>0.9999	0.4737	>0.9999	0.2308
	Severe	0.0931	0.3008	0.4737	>0.9999	>0.9999

Table 27: Comparison of rates of solicited adverse events between Groups as measured for 7 days following each vaccination. Rates of occurrence were compared by Fisher's Exact test and the respective p-values are shown. Comparisons with a p-value <0.05 are highlighted in green.

	1st Vaccination		2nd Vaccination		3rd Vaccination	
	n (%)	*p*	n (%)	*p*	n (%)	*p*
Group 1 & 2	10 (50)	0.0014	14 (70)	0.2351	11 (57.9)	0.5145
Group 3 & 4	20 (95.2)		18 (90)		14 (70)	
Group 1					4 (44.4)	0.3698
Group 2					7 (70)	
Group 3					7 (70)	1
Group 4					7 (70)	

Table 28: Number of subjects with at least one report of unsolicited AE in the 28 day period post vaccination (Days 0-28). 2-tailed p value obtained using Fisher's exact test.

	1st Vaccination		2nd Vaccination		3rd Vaccination	
	n (%)	*p*	n (%)	*p*	n (%)	*p*
Group 1 & 2	0	1	2 (10)	0.6614	1 (5.3)	1
Group 3 & 4	1 (4.8)		4 (20)		1 (5.0)	
Group 1					1 (11.1)	0.4737
Group 2					0	
Group 3					1 (10)	1
Group 4					0	

Table 29: Number of subjects with at least one report of a grade 3 unsolicited AE in the 28 day period post vaccination (Days 0-28). 2-tailed p value obtained using Fisher's exact test.

	1st Vaccination		2nd Vaccination		3rd Vaccination	
	n (%)	*p*	n (%)	*p*	n (%)	*p*
Group 1 & 2	10 (50)	0.0203	11 (55)	0.176	7 (36.8)	0.1128
Group 3 & 4	18 (85.7)		16 (80)		13 (65)	
Group 1					3 (33.3)	1
Group 2					4 (40)	
Group 3					7 (70)	1
Group 4					6 (60)	

Table 30: Number of subjects with at least one report of an unsolicited AE deemed possibly, probably or definitely related to vaccination in the 30 day period post vaccination (Days 0-28). 2-tailed p value obtained using Fisher's exact test.

			Timepoint at which first identified			
			Post-Vaccine 1	Post-Vaccine 2	Post-Vaccine 3	Post-CHMI
Group	**AE**	**Max severity**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
1	**Anaemia**	**Mild**				1 (12.5)
	Eosinophilia	**Mild**		1 (10)		
	Hypokalaemia	**Mild**	1 (10)			
	Lymphopenia	**Mild**			1 (11.1)	
		Moderate				1 (12.5)
		Severe				1 (12.5)
	Raised ALT	**Mild**	1 (10)	1 (10)		
	Uraemia	**Mild**	1 (10)			
2	**Anaemia**	**Mild**				1 (11.1)
	Hypokalaemia	**Mild**				1 (11.1)
		Moderate	1 (10)		1 (10)	1 (11.1)
	Leukocytosis	**Mild**				1 (11.1)
	Lymphopenia	**Moderate**				1 (11.1)
	Raised ALT	**Mild**				1 (11.1)
		Moderate	1 (10)			
	Thrombocytopaenia	**Moderate**	1 (10)			
3	**Anaemia**	**Mild**				2 (20)
	Eosinophilia	**Moderate**		1 (10)		
	Hypokalaemia	**Mild**				1 (10)
		Moderate				1 (10)
	Lymphopaenia	**Mild**				1 (10)
		Severe				3 (30)
	Neutropaenia	**Mild**				2 (20)
	Raised ALT	**Moderate**			1 (10)*	
		Severe				1 (10)
4	**Anaemia**	**Mild**	1 (9.1)			
		Moderate		1 (10)		
	Hypokalaemia	**Mild**		1 (10)		
	Leukopenia	**Mild**	1 (9.1)			
	Lymphopaenia	**Moderate**	1 (9.1)*	1 (10)	1 (10)*	
	Neutropaenia	**Mild**	3 (27.2)		1 (10)	1 (11.1)
	Raised ALT	**Mild**	1 (9.1)			
5	**Hyperbilirubineamia**	**Moderate**				1 (25)
	Hypokalaemia	**Mild**				1 (25)
	Lymphopaenia	**Severe**				1 (25)

Table 31: Table of all laboratory adverse events identified throughout the VAC059 trial, and the timepoint at which the abnormality was first identified. The table shows all events in which a laboratory value has deviated by at least 1 degree of severity from the baseline (screening) value as defined in the laboratory adverse event

grading tables that can be found in the appendix. *denotes events at which the laboratory value lies within the moderate adverse event range, but was in the mild adverse event range at screening. ALT = Alanine aminotransferase

4.7.3.37. Unsolicited adverse events

Verbatim reports of unsolicited signs and symptoms were reviewed by a physician and coded and tabulated according to the MedDRA Dictionary for Adverse Reaction Terminology (See safety appendix for full listings of unsolicited AEs). Each unsolicited AE was annotated with a causality grading according to likely relationship to vaccination of either Unrelated, Unlikely, Possible, Probable or Definite. The numbers of subjects with at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported up to 28 days (Day 0-Day 27) after vaccination have been tabulated and are shown in Table 28. The same tabulation has been performed for grade 3 unsolicited AEs (Table 29) and for unsolicited AEs with a relationship to vaccination (Table 30). There was no difference between groups in rates of severe unsolicited AEs following any of the vaccinations. There was a higher frequency of AEs related to vaccination in Group 3&4 than Group 1&2 following the first vaccination only (85.7% vs 50%, p=0.0203). There were no significant differences between groups after the second and third vaccination.

4.7.3.38. Clinical laboratory evaluation

Laboratory AEs following vaccination and CHMI are summarized in Table 31. Laboratory AEs were infrequent following vaccination in this study. The only severe laboratory AEs in this trial were recorded following CHMI (5 episodes of severe

lymphopenia and 1 episode of severe transaminitis, all of which fully resolved without sequelae). Three female subjects developed anaemia (2 subjects grades as mild, normocytic, 1 subject graded as moderate, microcytic at final study visit) during the course of the trial, with a likely diagnosis of mild iron deficiency. All 3 subjects were asymptomatic. They were advised to visit their GPs for follow up. There were no other ongoing laboratory AEs of note at study end.

4.7.3.39. Sporozoite Challenge

36 vaccinated subjects (Groups 1, 2, 3 and 4), and 4 unvaccinated infectivity control volunteers (Group 5) underwent sporozoite challenge by the bite of *Anopheles stephensi* mosquitos infected with the 3D7 strain of *P. falciparum*.

Safety of vaccinated subjects following CHMI from other studies of malaria vaccines, including some used in this trial, have been previously described[159], and a detailed analysis of solicited symptoms post-CHMI has not been performed for this thesis. However, no unexpected adverse events or clinical signs of immunopathology were observed in vaccinees following CHMI. No SAEs related to CHMI occurred, and there were no serious or untoward medical occurrences following CHMI.

4.7.4. Immunogenicity

4.7.4.40. Humoral response to vaccination

Antibody responses against the NANP repeats and C-terminal regions of the circumsporozoite protein (CSP) as well as the full-length protein were measured by enzyme-linked immunosorbent assay (ELISA). Responses were measured at baseline and key time points after each vaccination, and additionally at day 42, and 35 and 90

days after CHMI for NANP ELISA. The NANP-specific response over time is shown in (Figure 41) for all volunteers receiving RTS,S/AS01B alone (groups 1 and 2 combined) and volunteers receiving RTS,S/AS01B with viral vectors (groups 3 and 4 combined) until C-1, whereupon groups 2 and 4 received a fractional third dose of RTS,S/AS01B and are shown separately. In some further analyses, results from groups 1 and 2 (RTS,S/AS01B only), and groups 3 and 4 (RTS,S/AS01B with viral vectors) have been combined as there were no significant differences between these groups on these assays. Peak NANP IgG were measured at day 42 in all groups with a subsequent decline by day 56. In the groups receiving RTS,S/AS01B alone responses were re-boosted by the third vaccination but this did not occur in the groups that had received RTS,S with viral vectors (Figure 41). There was no significant difference in NANP IgG titres between the RTS,S alone and RTS,S plus viral vectors groups after first vaccination (Figure 42). This was followed by a trend to higher titres in the RTS,S/AS01B only group after second vaccination (P=0.06) and significantly higher titres in this group after third vaccination (P=0.0007). Similarly, C-terminal IgG titres and antibodies against full-length CS protein were comparable between groups after the initial vaccination but were significantly lower in the RTS,S/AS01B plus viral vectors group after the second and third vaccinations (Figure 43 P=0.007 and P=0.0005, respectively for C-terminal IgG; Figure 22 P=0.03 and P=0.001 respectively for full-length CS protein IgG).

Total IgG antibody avidity against full-length CSP was measured using a sodium thiocyanate (NaSCN) displacement ELISA at day 28, day 56 and C-1 (Figure 44), and of total IgG, IgG1 and IgG2 against NANP at C-1 (Figure 46). There was a significant increase in the avidity of CS-specific IgG after the second vaccination, but this was

not further increased by a third dose in any vaccination group. CS IgG avidity did not differ significantly between groups at any time point, and there were no significant differences in the avidity of NANP-specific total IgG, or IgG2 between groups at C-1. NANP-specific IgG1 avidity at C-1 was significantly higher in group 2 when directly compared with group 1 (Mann-Whitney analysis, P=0.005) but there were no significant differences in a comparison of all groups (Kruskal-Wallis with Dunn's correction P=0.0837).

Antibody responses against TRAP were also measured by in groups 3 and 4 only. There were no significant differences between group 3 and group 4 when comparing TRAP antibody responses at any timepoint. Titres from D0, D28, D35, D42, D56, D63, C-1, C+35 and C+90 were used to compare a time course with the VAC055 trial (Chapter 3) where RTS,S/AS01B and viral vectors were administered two weeks apart from each other (Figure 47). A key difference between the trial presented in this chapter, and the VAC055 trial presented in Chapter 3 is the additional MVA-ME-TRAP boost administered to Group 3 and 4 subjects at week 4. Peak TRAP IgG levels were measured at day 42, 2 weeks after the initial MVA vaccination and were not re-boosted by the second MVA 4 weeks later. Peak pre-boost TRAP-specific IgG titres measured at day 28 (28 days after ChAd63 vaccination) were comparable to those in the staggered regimen (measured at 21 days after ChAd63 vaccination). Peak titres at day 42, 2 weeks after the first MVA vaccination in the concomitant regimen (VAC059) were comparable to those after a single MVA boost given at 8 weeks in the staggered regimen (VAC055), however, peak titres after the second MVA, measured at C-1 in the concomitant regimen (VAC059) were significantly lower than those at

the same time point in the staggered regimen (VAC055; P=0.03). There were no significant differences in the titres measured after CHMI between the two regimens.

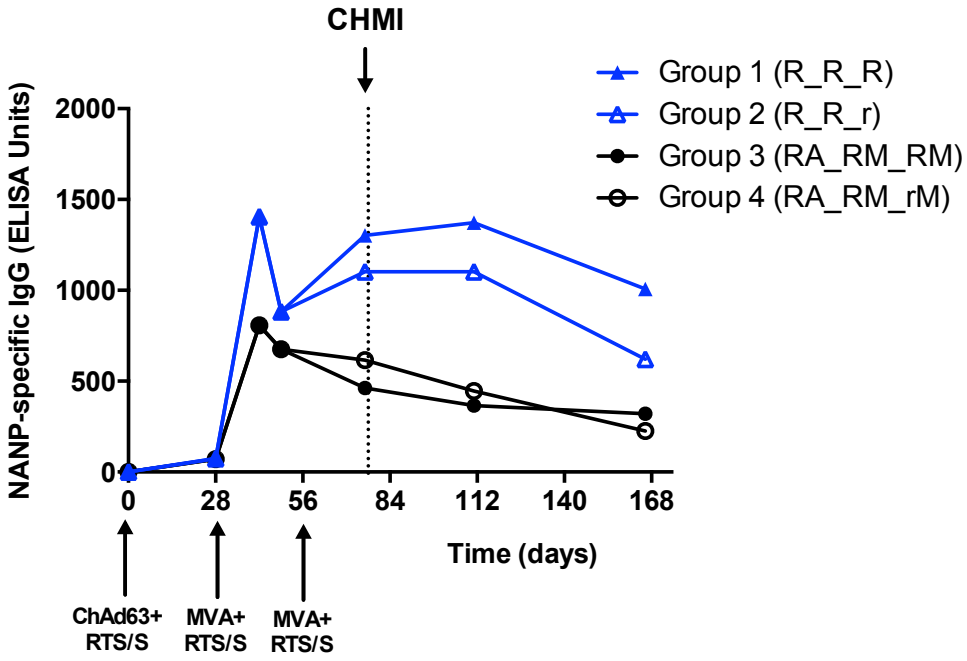


Figure 41: Median NANP IgG time courses.

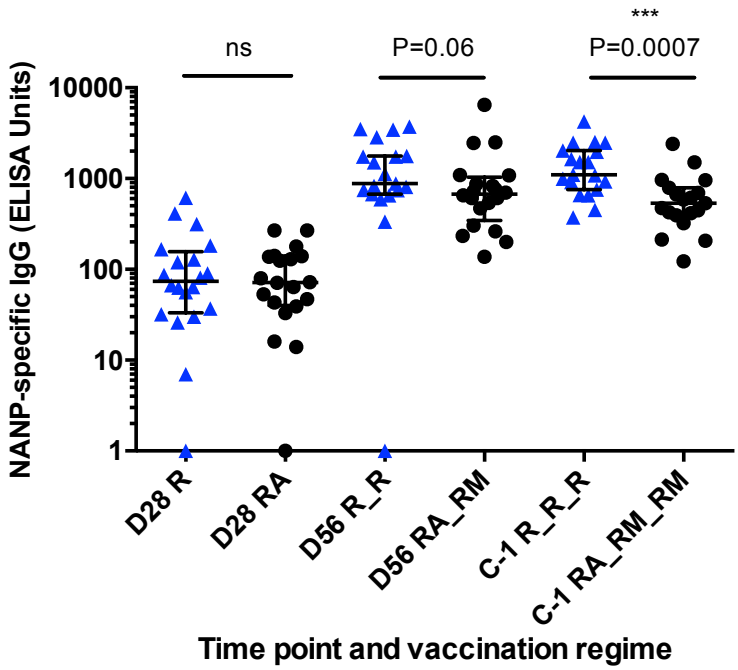


Figure 42: NANP IgG responses after 1st RTS,S vaccination (day 28, D28), after 2nd (day 56, D56) and 3rd (C-1) RTS,S vaccinations, for volunteers receiving RTS,S/AS01B alone or RTS,S/AS01B and viral vectors coadministered. 2-tailed Mann-Whitney analyses. A trend to higher NANP IgG in RTS,S alone group was observed after 2nd vaccination P=0.06 and significantly higher titres after 3rd vaccination P=0.0007 (***)

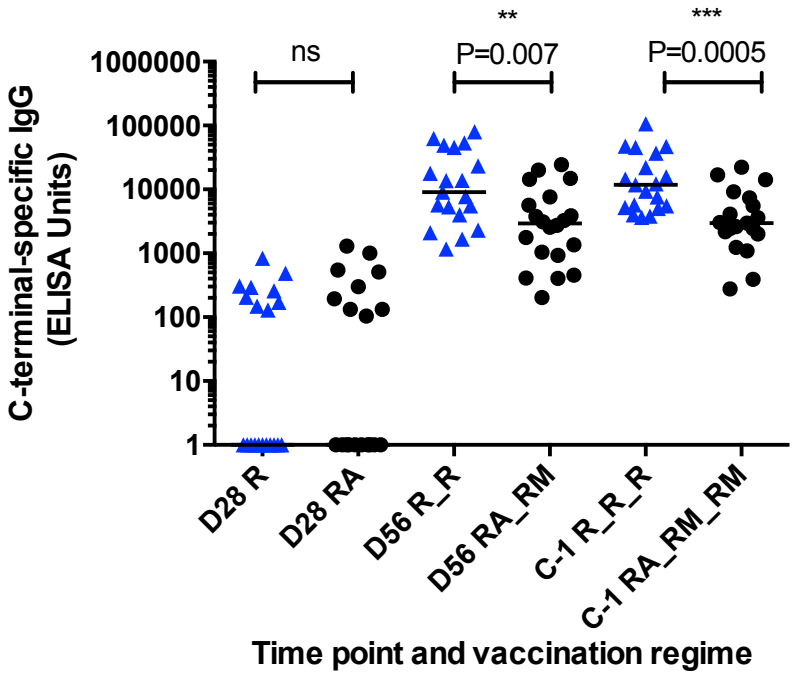


Figure 43: C-terminal IgG responses after each RTS,S vaccination. Significantly higher titres in RTS,S only group after 2nd and 3rd vaccinations, 2-tailed Mann-Whitney, 0.007 (**) and 0.0005 (***) respectively.

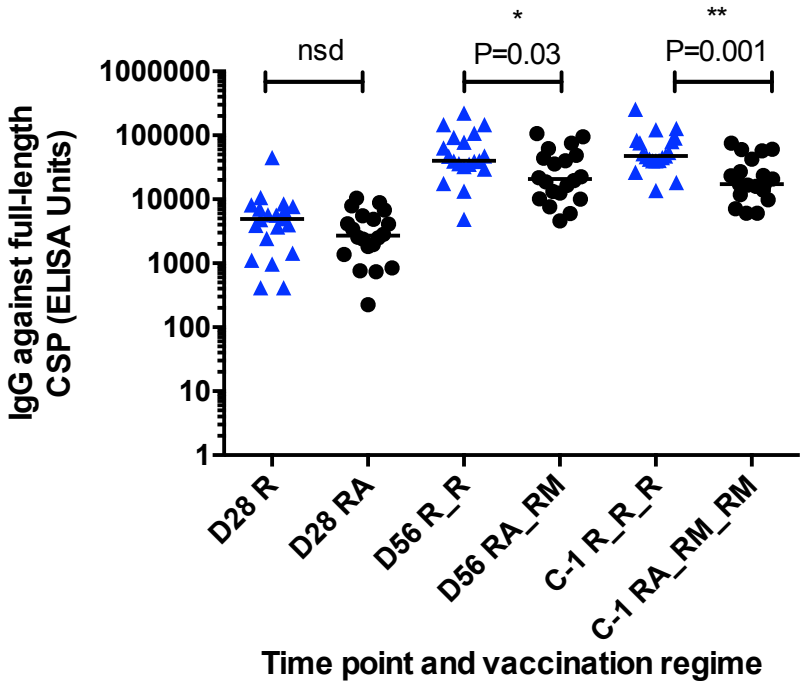


Figure 44: IgG responses against full-length CSP. Significantly higher titres in RTS,S only group after 2nd and 3rd vaccinations, 2-tailed Mann-Whitney P= 0.03 (*) and 0.001 (**) respectively.

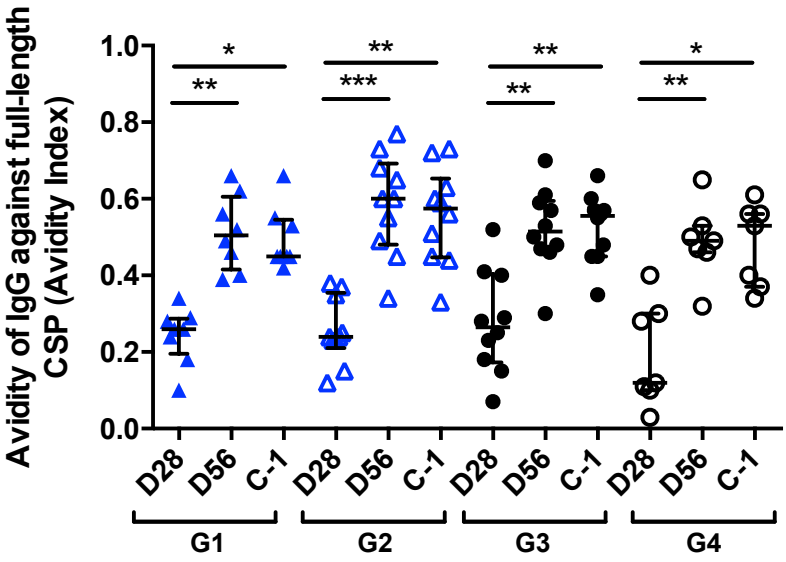


Figure 45: Avidity of total IgG against full-length CSP in each group at D28, D56 and C-1. Friedman test with Dunn's correction for comparison of time points within each group P value = 0.0009 (***), <0.0001 (****), <0.0001 (****), 0.003 (**) for G1, 2, 3 and 4 respectively. No significant difference between groups at any time point, Kruskal-Wallis analysis with Dunn's correction.

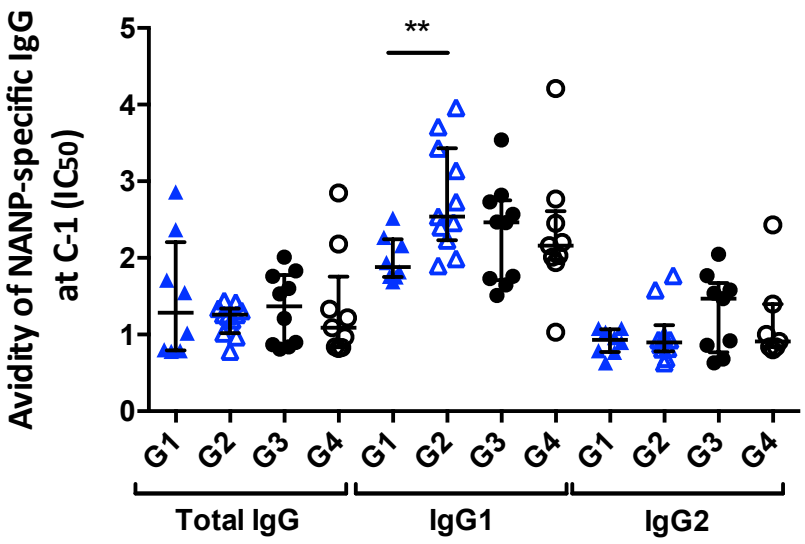


Figure 46: Avidity of total IgG, IgG1 and IgG3 against NANP for G1, 2, 3 and 4. No significant differences between groups for total IgG or IgG2 avidity. Significantly higher IgG1 avidity in G2 compared with G1, 2-tailed Mann-Whitney, P value = 0.007 (**), no significant differences between other groups or isotypes.

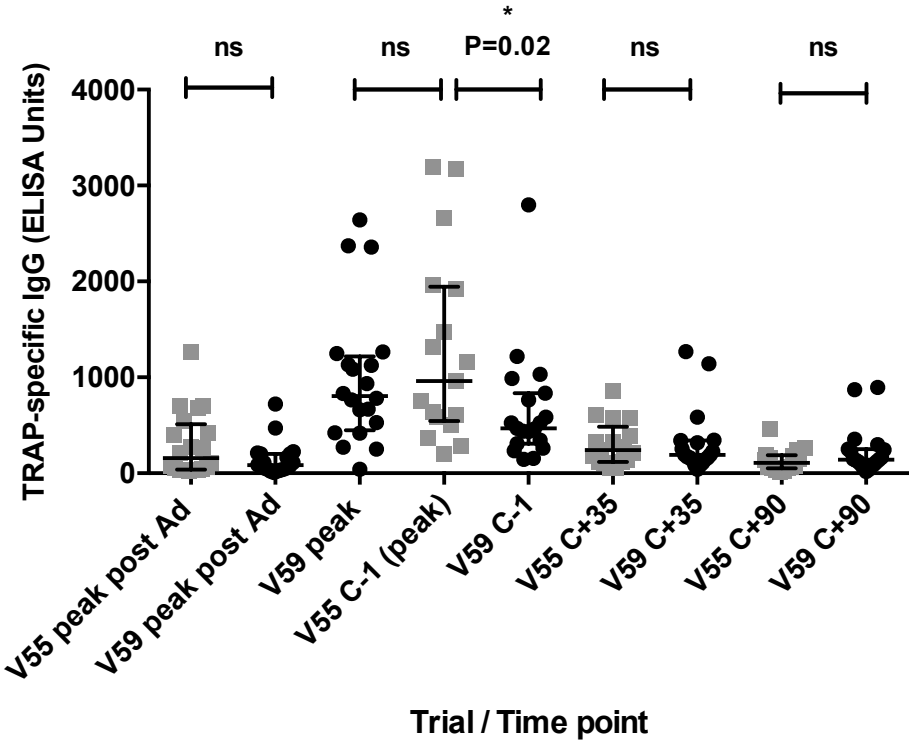


Figure 47: TRAP-specific IgG responses. RTS,S coadministered with vectors (VAC59 G3/4, black circles), RTS,S and vectors administered at a 2 week interval (VAC55 G1, grey squares). No significant difference between trials at peak post ChAd vaccination (21 days after ChAd), 35 days post challenge (C+35) or 90 days post challenge (C+90) and peak post MVA vaccinations compared between trials, 2-tailed Mann-Whitney. No

significant difference between peak post-MVA in VAC55 (C-1) and peak post-1st MVA in VAC59 (D42), significantly lower TRAP IgG titres at peak post-2nd MVA in VAC59 (C-1), 2-tailed Mann-Whitney P= 0.02 (*) respectively.

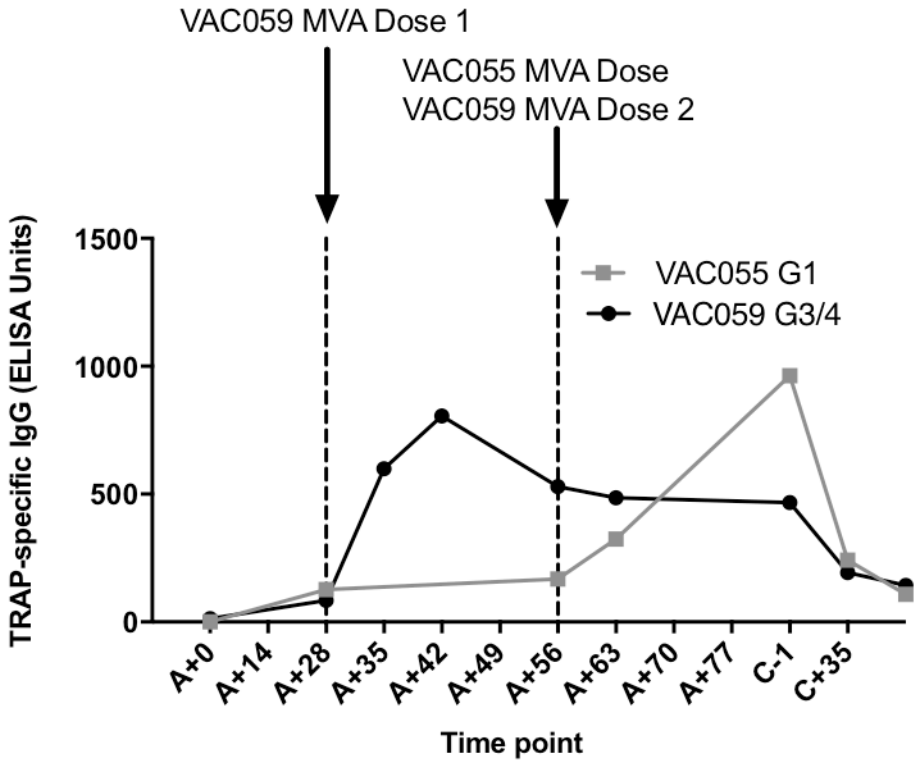


Figure 48: Median TRAP IgG time courses in VAC055 (grey squares) and VAC059 (black circles). R, full dose RTS,S/AS01B; A, ChAd63 ME-TRAP; M, MVA ME-TRAP; r fractional dose RTS,S/AS01B; CHMI, controlled human malaria infection; C-1, day before CHMI; SFCs, spot-forming cells per million PBMC; ns, not statistically significant.

4.7.4.41. Anti-vector antibody responses

Anti-vector antibodies against the MVA WR113/D8L protein were measured by total IgG ELISA in groups 3 and 4 at baseline and after each subsequent MVA vaccination (day 42, and C-1). (Figure 49). One subject was strongly positive for anti-MVA antibodies at baseline, and two volunteers were borderline positive, although none had received prior smallpox vaccination. There was a significant increase in anti-MVA

IgG titres after the first MVA vaccination and these were boosted by the second vaccination, by which point, all volunteers were seropositive (Kruskal-Wallis analysis with Dunn's correction P<0.0001). The increase in anti-vector antibodies after initial MVA vaccination at 4 weeks was comparable to that induced by a single MVA given as given in the VAC055 trial (Chapter 3; Figure 50), however, there was a significant increase in the fold-change in anti-MVA titres after two MVA doses (Kruskal-Wallis analysis with Dunn's correction P=0.0002). When comparing protected subjects with non-protected subjects, anti-MVA titres at baseline and C-1 were comparable, but titres measured at day 42, 2 weeks after the initial MVA vaccination, were significantly higher in non-protected volunteers (Figure 51, P=0.02). There was a trend towards a negative association between anti-MVA IgG after the initial MVA (D42) and TRAP-specific T cell responses after the second MVA (C-1) (Figure 52, r=-0.6333, P=0.08). There was no association between TRAP-specific T cell responses and anti-MVA antibodies at day 42 (Figure 52) or between anti-MVA antibodies at C-1 and TRAP-specific T cells at C-1 after a single MVA administered at 8 weeks in the VAC055 trial (Chapter 3; Figure 53).

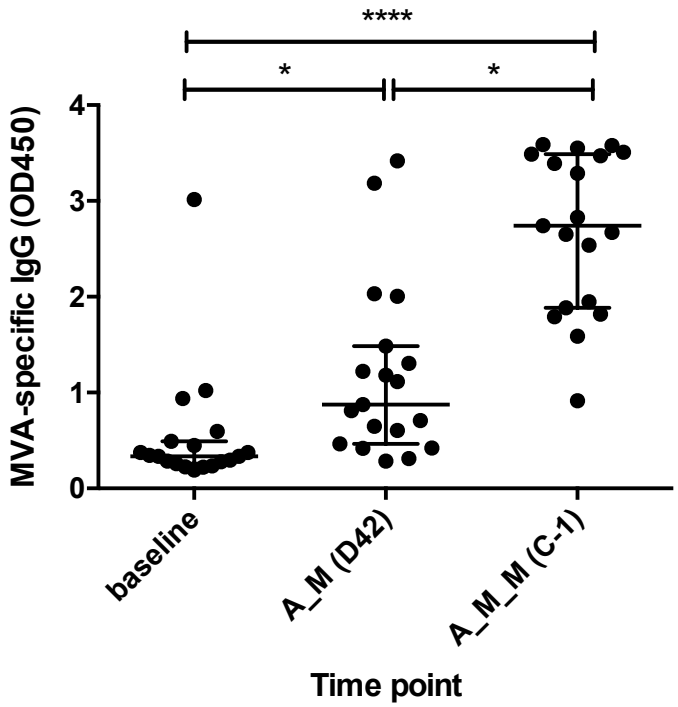


Figure 49: Total IgG against MVA at baseline (D0), after 1st MVA vaccination (D42) and after 2nd MVA vaccination (C-1) for G3/4. Kruskal-Wallis with Dunn's correction, * p<0.05, **** p<0.0001.

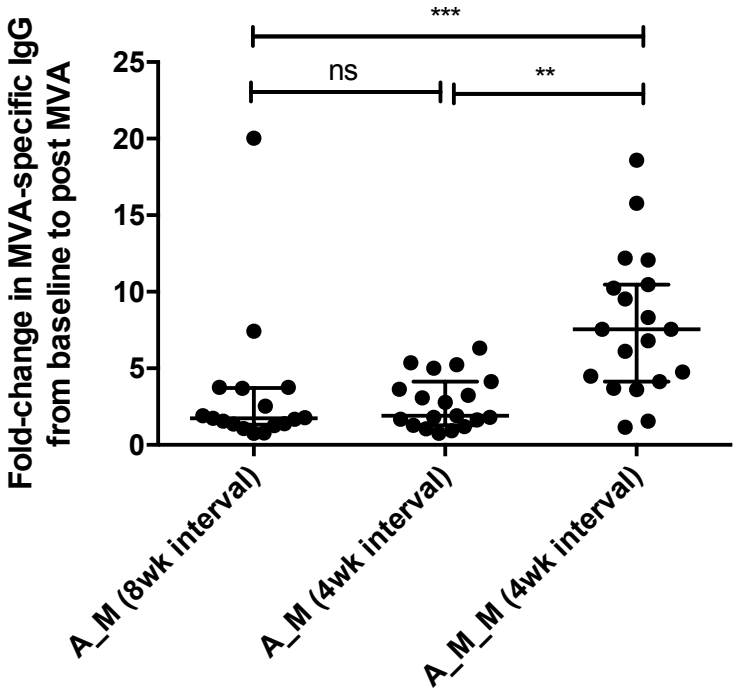


Figure 50: Fold-change in anti-MVA antibody from baseline to peak post-MVA in VAC055 (MVA administered 8 weeks after ChAd63, A_M), peak post-1st MVA in VAC059 G3/4 (D42, A4M) and peak post-2nd MVA in VAC59 G3/4 (C-1, A4M4M), Kruskal-Wallis with Dunn's correction p=0.002 (***).

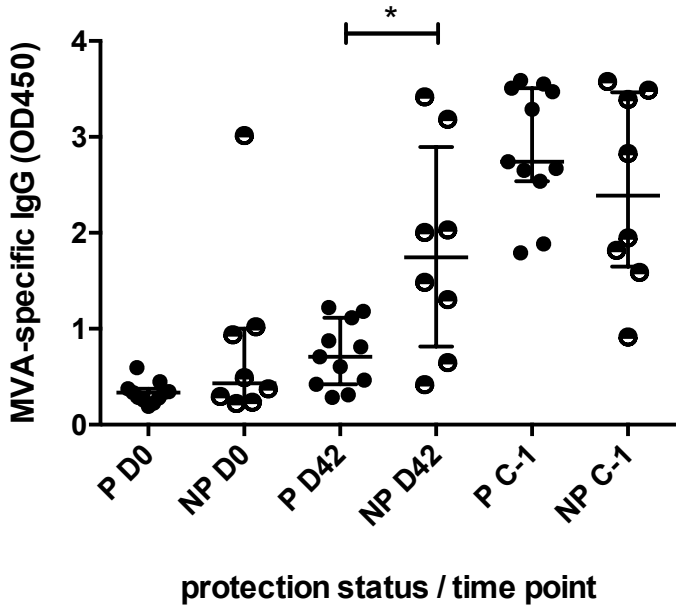


Figure 51: Anti-MVA IgG responses at baseline, post-1st and post-2nd MVA in volunteers protected (P) and non-protected (NP) after CHMI. 2-tailed Mann-Whitney comparison between groups at each time point, * p=0.02 at D42.

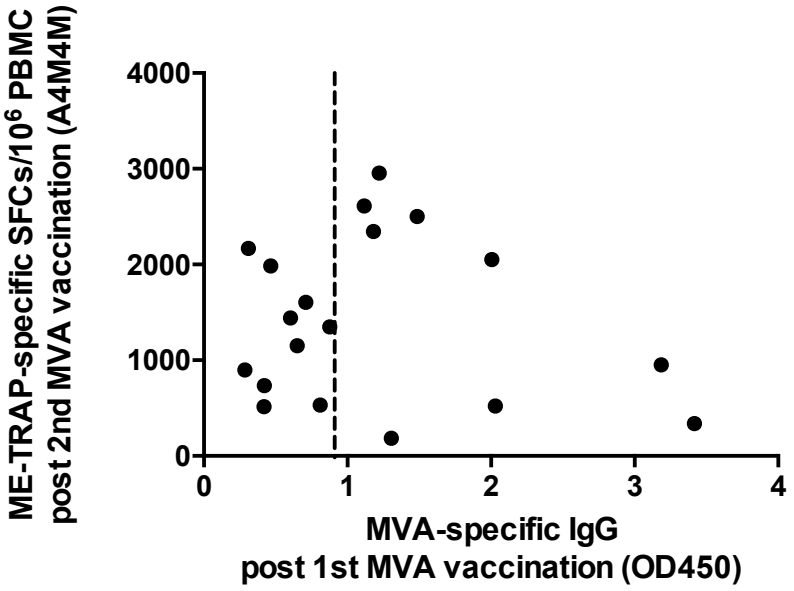


Figure 52: Relationship between anti-MVA antibodies at peak post-1st MVA (D42) and ME TRAP-specific T cell responses post-2nd MVA at C-1 in VAC059 G3/4, Spearman's r = -0.63, p=0.07.

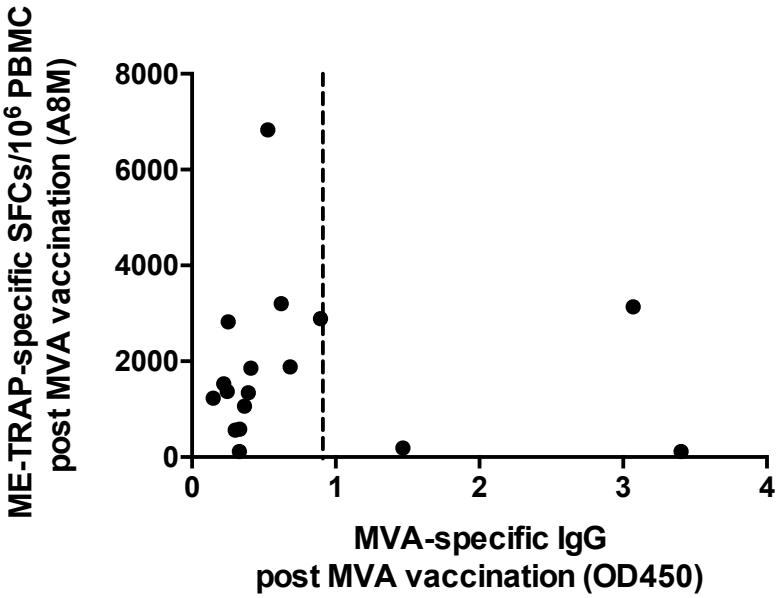


Figure 53: Relationship between anti-MVA antibodies at peak post MVA (C-1) and TRAP-specific T cell responses post MVA at C-1 in VAC055 G1, spearman's r = 0.17, p=0.5.

4.7.4.42. Cellular response to vaccination

Ex-vivo interferon γ (IFNγ) T-cell responses to CSP were measured by enzyme-linked immunosorbent spot (ELISPOT) assay in all groups at baseline, and after vaccination. CSP-specific T cell responses peaked 2 weeks after the second dose of RTS,S (Day 42) in all groups (Figure 54) with a median of 89 spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMC), interquartile range (IQR) 31-156 SFC. There were no significant differences between any individual vaccine groups (p=0.75, Kruskal-Wallis test), or between pooled groups that received the fractional third dose (Groups 1 & 3) of RTS,S/AS01B compared with the full dose (p=0.5, 2-tailed Mann-Whitney test comparing groups 1 and 3 with groups 2 and 4). After the peak, responses across all four groups decreased significantly from day 42 to a median of 28 SFC (IQR 12-117 SFC) at C-1 (p=0.009, 2-tailed Wilcoxon test).

T cell responses to ME-TRAP were measured by ex-vivo ELISPOT in groups 3 and 4 only and are shown in Figure 55. Peak responses were measured at Day 35, one week after the first dose of MVA ME-TRAP with a median of 2531 SFC (IQR 1949-4042). This high level of cellular immunogenicity is comparable to that previously observed after an eight-week prime-boost interval with ChAd63 and MVA ME-TRAP (2436 SFC, IQR 1064-3862 (Figure 56).[79] Although T-cell responses were re-boosted following administration of a second dose of MVA, the levels observed were significantly lower than after the first MVA dose (2531 SFC, IQR 1949-4042 after first MVA compared with 901 SFC, IQR 509-1910, p=0.04, one-way ANOVA).

Flow cytometry was used in conjunction with intracellular cytokine staining (ICS) to further identify the phenotype of T cell response. Expression of IFNγ, interleukin-2 (IL2) and tumour necrosis factor-α (TNFα) by $CD4^+$ and $CD8^+$ T cell subsets was measured. Cytokine responses did not differ significantly between groups to CSP peptides at either day 42 or C-1 and were predominantly from $CD4^+$ T cells (Figure 57). In contrast, ICS responses to TRAP peptides as measured in groups 3 and 4 subjects only, demonstrated expression of cytokines from similar proportions of $CD4^+$ and $CD8^+$ T cells (Figure 58). The majority of $CD8^+$ T cell responses to TRAP were found to express a cytokine profile that contained IFNγ (Figure 59). A substantially reduced proportion of monofunctional cells expressing IFNγ were observed following the second dose of MVA at Day 63 and C-1, but not after ChAd63 or the first dose of MVA. Monofunctional $CD8^+$ T cells expressing IFNγ are a subset of cells that have previously been shown to be associated with vaccine-induced protection against malaria. [79]

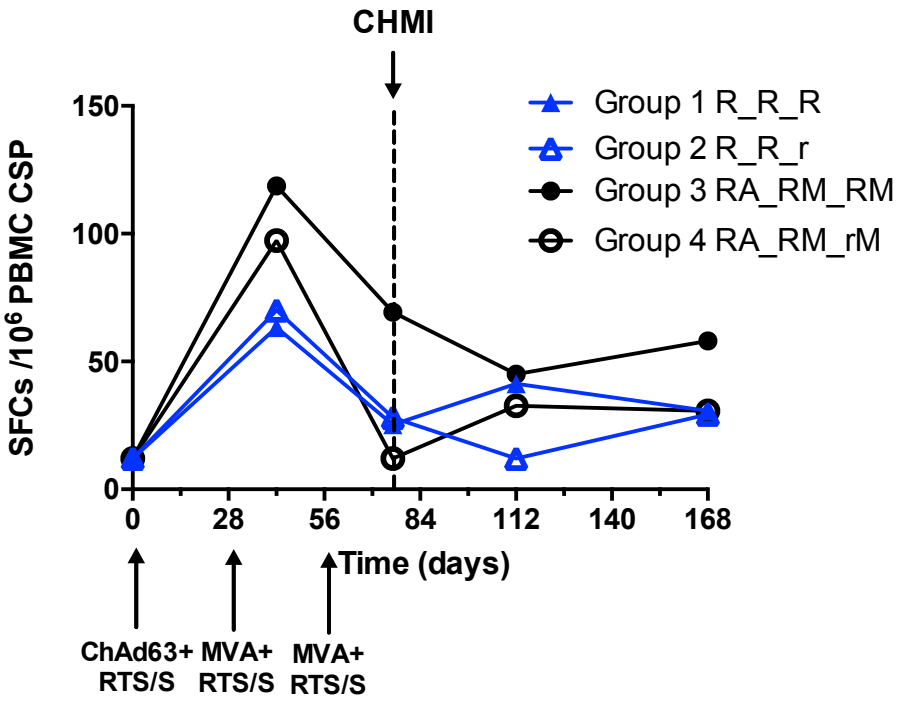


Figure 54: Median specific T-Cell response to summed circumsporozoite protein (CSP) peptide pools as measured by IFNγ- ELISPOT. R, full dose RTS,S/AS01B; A, ChAd63 ME-TRAP; M, MVA ME-TRAP; r fractional dose RTS,S/AS01B; CHMI, controlled human malaria infection; SFCs, spot-forming cells per million PBMC

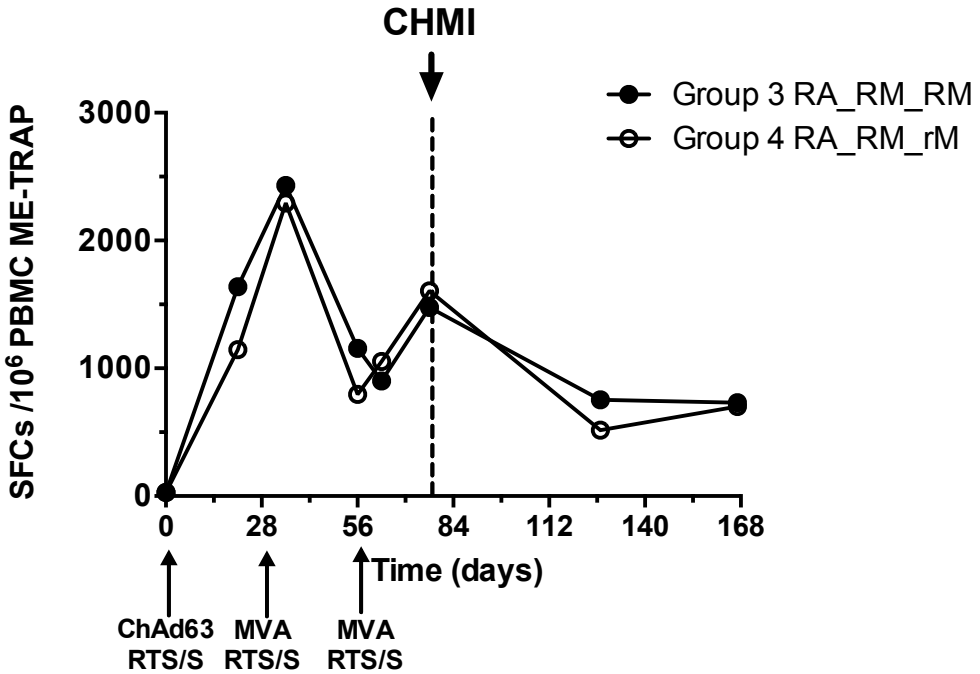


Figure 55: Median specific T-Cell response response to summed peptide pools for ME-TRAP as measured by IFNγ- ELISPOT..

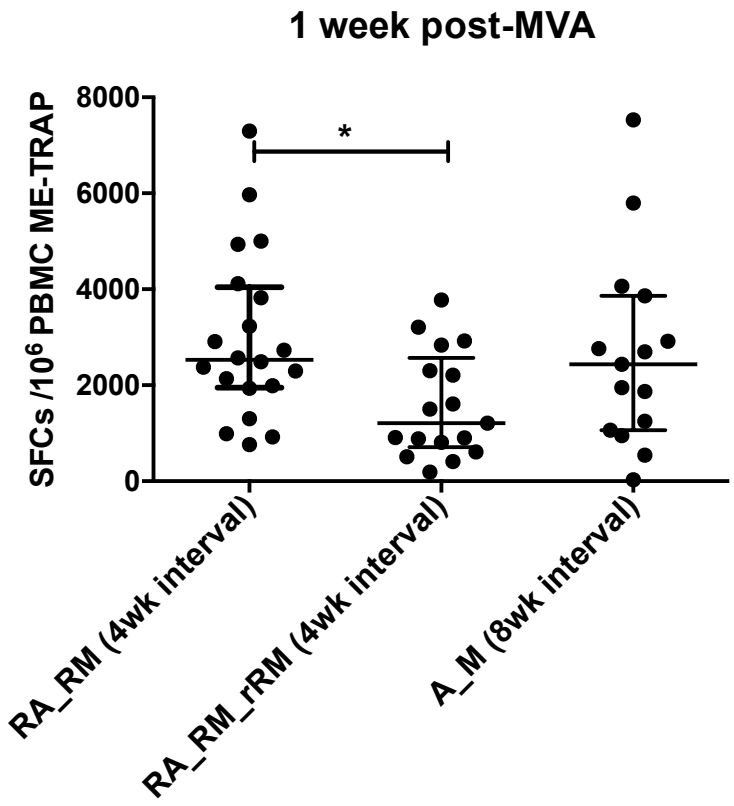


Figure 56: ME-TRAP-specific T cell responses one week after one or two doses of MVA co-administered with RTS,S/AS01B, *, P=0.04 one-way ANOVA.

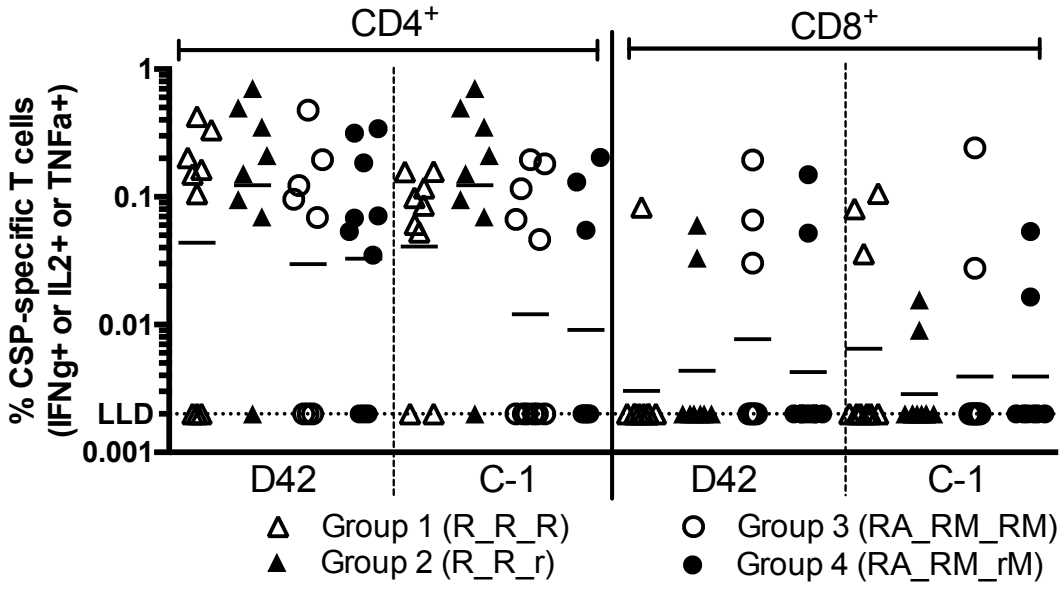


Figure 57: ICS responses to CSP peptides spanning the length of the vaccine antigen

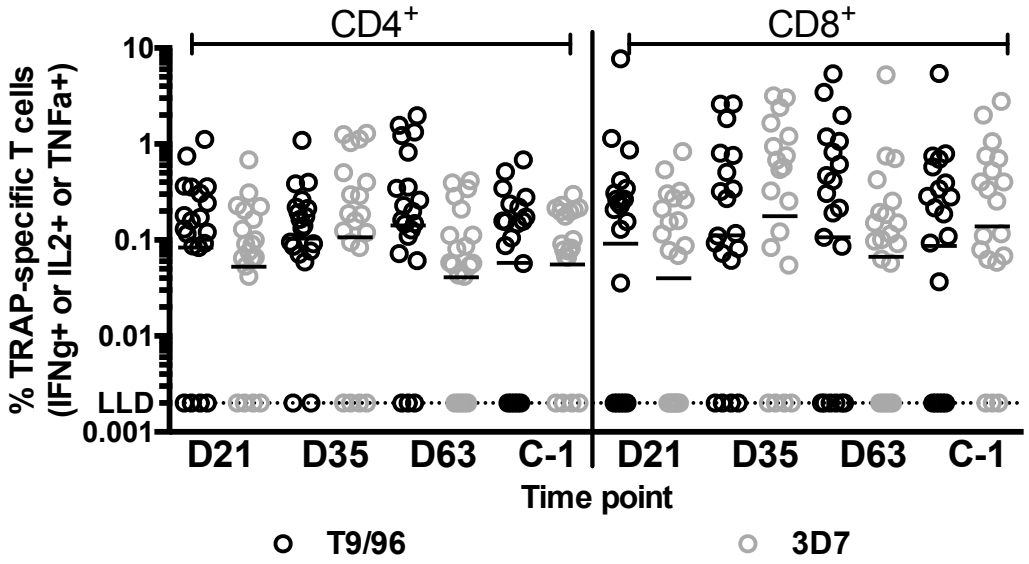


Figure 58: ICS responses to TRAP peptides from either the T9/96 or 3D7 strain of *Plasmodium falciparum*. Groups 3 and 4 are combined as both received identical doses of viral vectors encoding ME TRAP. C-1, one day prior to controlled human malaria infection; LLD, lower limit of detection.

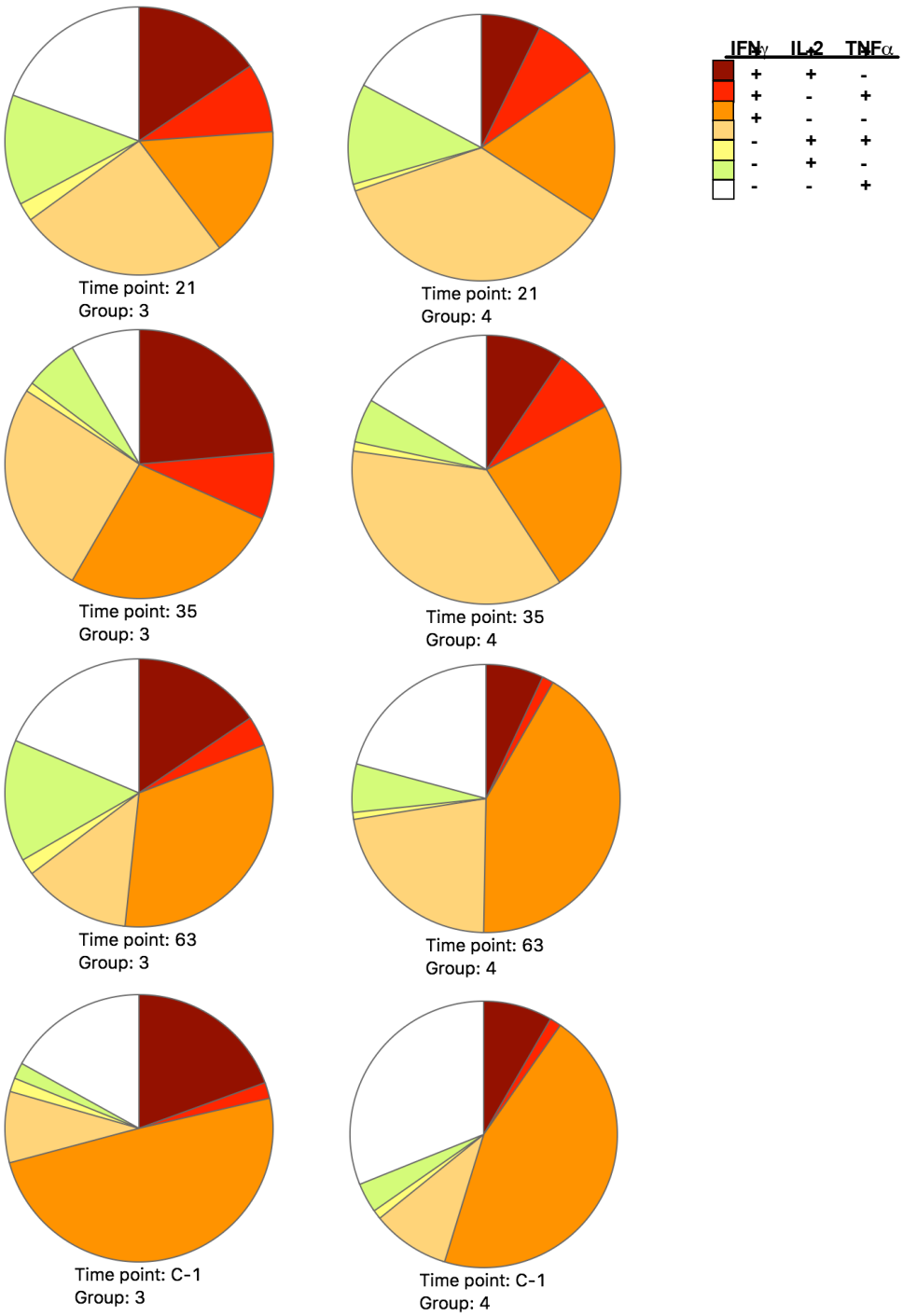


Figure 59: CD8+ T cell responses to TRAP were dominated by cells expressing IFNg either alone or in combination with other cytokines. Group 3 tended to have more triple-positive cells than group 4. Responses did not change substantially between post-Ad and post-first MVA, but the 2nd dose of MVA substantially reduced the proportion of monofunctional cells expressing IFNg (D63 and C-1).

4.7.4.43. Contribution of TRAP-specific T cells to efficacy in RTS,S plus viral vectors groups.

In the groups that received vaccination with RTS,S/AS01B alone (groups 1 and 2) 94% of subjects achieved sterile protection (SP) with a titre above 450 EU, 100% were SP with a titre above 1600EU, and some subjects were SP with titres as low as 430 EU. (Figure 60) IgG titres against CSP at C-1 were significantly reduced in subjects exhibiting SP in the groups that received RTS,S/AS01B with viral vectors when compared with those who received RST,S/$AS01_B$ alone (p=0.009, 2-tailed Mann-Whitney test, Figure 61). In groups 3 and 4 only a single subject reached the threshold of 1600 EU required for 100% SP with RTS,S alone, yet overall efficacy in the groups was 58% (11/19 SP, Figure 61). In order to achieve 100% SP in the RTS,S plus viral vectors groups, a CS antibody titre of at least 789 EU was required. In cases where subjects achieved SP with antibody titres below this level, substantial T cell responses to ME-TRAP were observed. In Groups 3 and 4, SP was not achieved with CS antibody titres below 700 EU without also having ELISPOT responses greater than 898 SFC, (Figure 62)

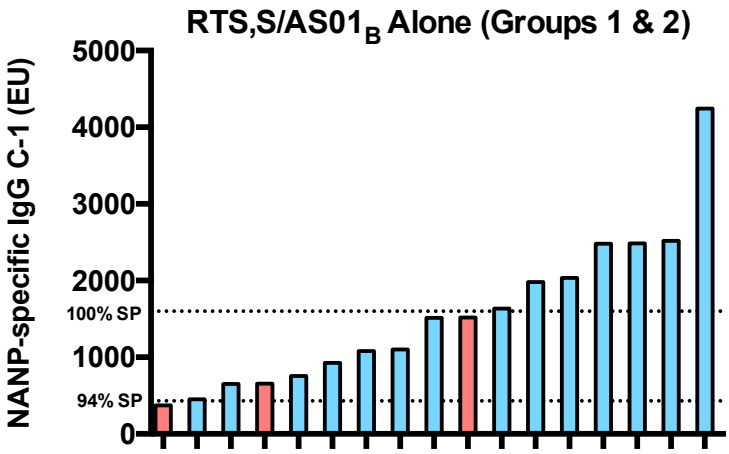


Figure 60: CS-specific IgG responses ordered by magnitude. Each bar represents a single volunteer. Pink bars, non-sterilely protected (NP); blue bars, sterilely protected (SP); EU, ELISA units. Dotted lines represent lowest level of CS IgG associated with SP (lowest SP, 430 EU) and level of CS IgG above which 100% SP was observed (1600 EU)

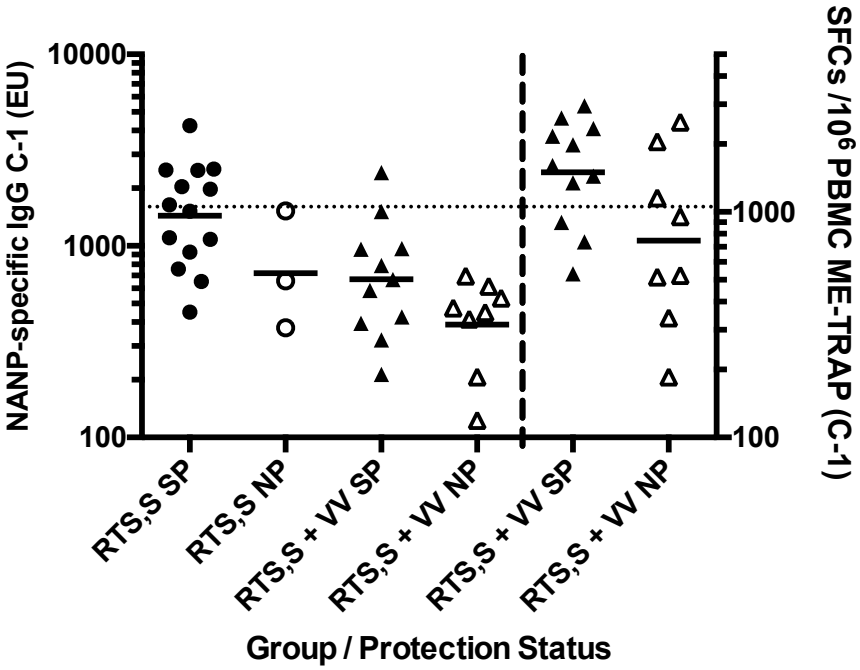


Figure 61: CSP-specific IgG titres (left y-axis) and TRAP-specific ELISPOT responses (right y-axis) at C-1, stratified by vaccine regime and CHMI outcome. **p=0.009, 2-tailed Mann-Whitney test. VV, viral vectors. Bars represent Geometric means.

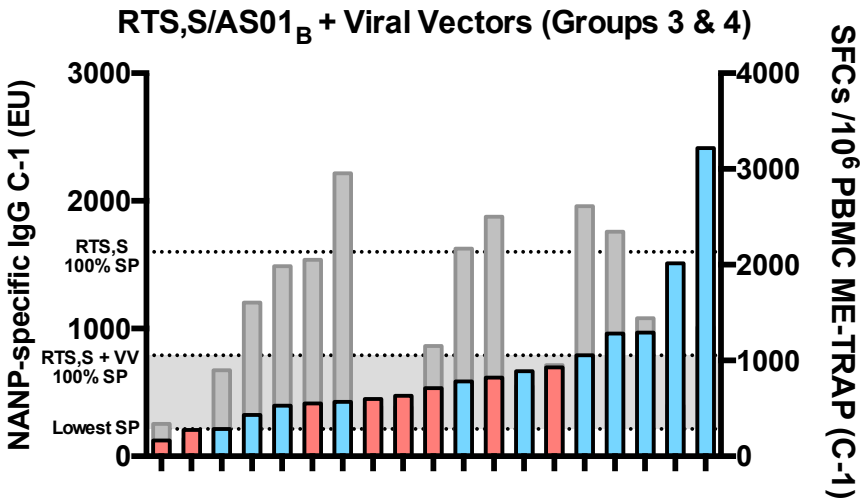


Figure 62: CS-specific IgG responses ordered by magnitude (left y-axis) and TRAP-specific ELISPOT responses (right y-axis) at C-1. Each bar represents a single volunteer. Pink bars, non-sterilely protected (NP); blue bars, sterilely protected (SP); grey bars, ELISPOT responses. Dotted lines represent lowest level of CS IgG associated with SP (lowest SP, 213 EU) and level of CS IgG above which 100% SP was observed with RTS,S alone (1600 EU) or in combination with viral vectors (789 EU). Shaded area represents range of IgG titres where T cell responses above 1000 SFC were observed in SP participants.

4.8. Discussion

This is now the second human clinical trial in which RTS,S/AS01B and viral vectors ChAd63 and MVA encoding ME-TRAP have been combined in the same vaccination schedule,[160] but the first in which viral vectors have been concomitantly administered with RTS,S/AS01B. This study was designed to include concomitant, same site administration on the basis that if high level efficacy had been observed, this could have supported the development of a mixed vaccine preparation, either to be supplied in a single vial, or mixed at the bedside. This would further simplify the supply and delivery of such a vaccine regimen, and mixed administration of RTS,S/AS01 with ChAd63/MVA ME-TRAP was supported by pre-clinical murine data

prior to this trial (Collins et al. Unpublished). This study shows that concomitant administration of these vaccines is safe, with no SUSARs, and no vaccine related SAEs in this trial. A higher frequency of AEs was observed in the groups that received the viral vectors in addition to RTS,S/AS01B, but the majority of AEs were mild in severity, and all were self-limiting.

In the VAC055 trial (Chapter 3), I reported a high level of vaccine efficacy (VE) in a group that received 3 standard doses of RTS,S/AS01B alone [VE 75% (95% CI 54-96)], and a group that received staggered administration of RTS,S/AS01B with ChAd63 and MVA encoding ME-TRAP [VE 82.4% (95% CI 64-100)].[160] In the study presented in this chapter, again, a high level of VE was observed in the groups that received only RTS,S/AS01B either at 3 full standard doses [VE 75% (95% CI 31.5-93.1)], or with a reduced third dose [VE 88.9% (95% CI 43.3-98.4)]. In the groups that received both viral vectors concomitantly with RTS,S/AS01B, observed VE was lower than in the RTS,S alone groups ([VE 60% (95% CI 25.3-82.7)] in the viral vectors plus 3 standard doses RTS,S/AS01B group and (VE 55.6% (95% CI 20.4-80.5)] in the viral vectors plus fractional third RTS,S/AS01B dose regimen). These results must be interpreted with caution, as no comparisons of VE between vaccinated groups is statistically significant, but they suggest that concomitant administration of these vaccines, according to the schedules and immunisation routes used in this trial, may negatively impact VE.

As with previous CHMI studies of RTS,S/AS01B[160-162], anti-CS Ab titres in this study were significantly higher in protected subjects immediately prior to CHMI (C-1) than in non-protected subjects. Anti-CS Ab titres reached peak levels at day 42; 14 days

after the second vaccination in both the pooled RTS,S alone groups and the RTS,S plus viral vectors groups. At the day 42 timepoint, there was no significant difference between any treatment groups. Of note, in the subjects who received RTS,S with viral vectors, the subsequent 3rd dose of RTS,S appears to be ineffective at boosting, or even maintaining anti-CS Ab titres, and the resulting anti-CS Ab titres on the day preceding challenge (C-1) were therefore significantly lower in these groups than in the RTS,S alone groups. There was no reduction in anti-CS Ab titres after co-administration of RTS,S with the ChAd63 vector, but titres were slightly reduced and significantly reduced after the first and second MVA respectively. This is suggestive that interference with induction of CS antibody responses may be associated more with the poxviral than the adenoviral vector.

Previous clinical trials of RTS,S and viral vector combination regimens, including that presented in Chapter 3 [94,160] have examined a schedule in which the dosing of RTS,S was separated from the dosing of viral vectors by an interval of at least 2 weeks. In the study presented here, at each vaccination, subjects in Groups 3 and 4 received a mixture of antigens that included the NANP repeats and T-cell epitopes of the CS protein, and the hepatitis B surface antigen in addition to multiple antigens on the surface of either the ChAd63 or MVA vectors. Following cellular invasion of the vector, peptides from the ME-TRAP insert will also be presented by MHC molecules on the surface of antigen presenting cells. With simultaneous exposure to multiple immunogenic antigens, there is a potential for each antigen to interfere with immune responses to the others. Immune interference may occur due to local chemical interactions, or immunological interactions, either local or systemic. In trials of non-malaria vaccines, it has been shown that immune interference is

complex and may result in enhancement or depression of responses to one or more antigens.[163] This may, in turn, impact on the resulting efficacy. In this trial, a significantly enhanced antibody response against the MVA vector to those seen in VAC055 (Chapter 3) was observed in groups 3 and 4, when comparing the responses after MVA 8 weeks following ChAd63 prime (Figure 50). This is possibly due to the additional dose of MVA given at week 4 in VAC059, or possibly due to the concomitant administration of the potent adjuvant AS01 at the same vaccination site. These factors did not appear to enhance MVA induced T cell responses against ME-TRAP, however. In addition, although a profound boosting effect of the 2nd vaccination on anti-CS Ab titres was observed in all the trial vaccination groups, the third dose of RTS,S/AS01B, when co-administered with MVA ME-TRAP failed to re-boost the antibody response to CSP. This, in conjunction with strong antibody responses to the MVA vector protein induced by the first MVA at four weeks, is suggestive that anti-vector immunity might have contributed to lack of boosting of CSP-specific antibodies after the 3rd dose. Furthermore, non-protected subjects had significantly higher anti-MVA Abs at day 42 (2 weeks after the first MVA at 4 weeks) than protective subjects (Figure 49), further implicating anti-vector immunity in the low efficacy observed in Groups 3 & 4. This striking observed immunological interference was unexpected, as pre-clinical murine studies had not found any detrimental impact of same site administration on immunogenicity or efficacy of the vaccine components (Collins et al. unpublished).

Further work is necessary to determine whether this apparent negative effect of concomitant administration can be overcome. Alternative solutions to evaluate would include concomitant administration but at different vaccination sites,

administering only a single dose of MVA ME-TRAP, or adjustments to the vaccine dose or formulation.

Another option would be to temporally separate the administration of the anti-sporozoite (RTS,S) and anti-liver-stage (ChAd/MVA ME-TRAP) vaccines based on the durable high efficacy of this approach observed in VAC055 (Chapter 3). The RTS,S/AS01 phase 3 trial showed highest immunogenicity and efficacy of this vaccine in 5-17 month olds, leading to the WHO Strategic Advisory Group of Experts recommending pilot implementation trials for older infants, which are now ongoing.[28] In contrast recent phase Ib data on ChAd63/MVA ME-TRAP vector administration in Gambian and Burkinabe infants [164,165] showed highest levels of T cell immunogenicity in younger age groups, specifically, 2-4 month old infants. This was despite the co-administration of standard EPI vaccines with the viral vectors. It is plausible, therefore that a malaria vaccine schedule in which viral vectors are separately administered to a subunit vaccine may offer a potentially deployable solution.

In populations living in high transmission settings, the strategic goals for malaria vaccine development include a vaccine suitable for completion of primary immunisation series by latest 9 months of age, with a possible booster dose at a later date, if necessary. In lower transmission settings, older children and adults may also be targeted.[166] RTS,S/AS01 is less immunogenic in babies aged 6-12 weeks than toddlers aged 5-17 months and anti-CS IgG titres are a surrogate of protection with reduced efficacy and durability also observed in the younger age-group[167]. This study shows, that in adults with anti-CS antibodies titres that may be suboptimal for

protection, as in groups 3 and 4, TRAP specific T cells induced by vaccination with viral vectors may contribute an additive element to VE. Specifically, the VE of 58% in the subjects who received RTS,S plus viral vectors (Groups 3 & 4) with CS antibody titres as low as in the non-protected RTS,S/AS01 only vaccinees (Figure 61) suggests an important additive effect of the viral vectors on overall efficacy. Further evaluation of co-administration of RTS,S/AS01 with viral vectors in a trial where lower VE of RTS,S/AS01 is predicted (eg. using a delayed CHMI model) could demonstrate this effect even more clearly than was possible with the study design employed here.

This is now the 3rd study to demonstrate a very high level of VE in subjects receiving a regimen that includes a fractional 3rd dose of RTS,S/AS01B.[24,168] It is the first to show this in a 0, 1, 2 month vaccine schedule. A recent study that evaluated a fractional 3rd dose regimen of RTS,S/AS01B, but one delivered on a 0, 1, 7 month schedule, concluded that the dose reduction was likely to be partially responsible for the increased somatic hypermutation in immunoglobulin genes and avidity that they observed in this group.[168] The investigators hypothesised that competitive antigen binding in the germinal centres led to survival and expansion of B cells, with surface immunoglobulins showing the highest antigen avidity. It was not possible to determine from this study, however, whether the fractional dosing was the main contributor to the high efficacy, or whether it was more attributable to the delayed boosting at 7 months. Two prior studies have evaluated the efficacy and immunogenicity of a 0, 1, 7-month schedule of RTS,S/AS01 compared to a 0, 1, 2-month schedule.[169-171] In the immunogenicity studies, conducted in Ghanaian children the authors concluded that peak humoral responses against CSP were

higher in the 0, 1, 2-month schedules, but area under the curve for anti-CS antibody responses were comparable. Conversely, peak CS-specific CD4+ T-cells were higher in the group that had received a 0, 1, 7-month schedule. In this efficacy study, conducted in children in 3 African countries, third dose spacing alone did not increase VE against clinical malaria.

In conclusion, no safety concerns arose from concomitant administration of ChAd63 and MVA viral vectors encoding ME-TRAP with RTS,S/AS01. Further work is required to evaluate the impact of concomitant administration, and the use of a fractional third dose of RTS,S/AS01 on vaccine efficacy.

Chapter 5

A Phase Ia, Dose-Escalating, Safety and Immunogenicity trial of the monovalent Zaire Ebola viral vector candidate vaccine ChAd3-EBO Z and the heterologous prime-boost candidate vaccine regimen ChAd3-EBO Z and MVA-BN® Filo in healthy UK adults (EBL01)

5. A Phase Ia, Dose-Escalating, Safety and Immunogenicity trial of the monovalent Zaire Ebola viral vector candidate vaccine ChAd3-EBO Z and the heterologous prime-boost candidate vaccine regimen ChAd3-EBO Z and MVA-BN® Filo in healthy UK adults (EBL01)

5.1. Authorship Statement

The work presented in this Chapter is part of a collaborative effort. Unless otherwise stated, all work was undertaken by me.

The study was designed in collaboration with staff at GlaxoSmithKline, Bavarian Nordic, and the National Institute of Health. The conduct of the trial was greatly assisted by the Jenner Institute Clinical Trials team (including Alison Lawrie, Rachel Roberts, Ian Poulton, Ruth Payne, Saranya Sridhar, Navin Venkatraman, Malick Gibani, Alice Minhinnick, Morven Wilkie, Felicity Hartnell, Oliver Griffiths, Raquel Lopez Ramon, Natalie Lella). This included some assistance with regulatory processes and clinical procedures (volunteer screening, follow up visits etc.).

Katie Ewer, Georgina Bowyer, Danny Wright, Tess Lambe, Nick Edwards, Kailan Sierra-Davidson and Jonathan Powlson performed the immunological assays, including the ELISAs, EliSPOTs and Flow Cytometry. Neutralising assays against live Ebolavirus were performed by Stephan Becker and team at the Institute of Virology, Philipps University Marburg, Germany.

Blood samples for haematology, biochemistry and serology (for HIV, Hepatitis B and C) were processed at the Trust laboratories at Oxford University Hospitals' NHS Trust.

Some of the data presented in this chapter has been published in the following articles:

- **Rampling T**, Ewer K, Bowyer G *et al*. A Monovalent Chimpanzee Adenovirus Ebola Vaccine - Preliminary Report. N Engl J Med. 2015 Jan 28. PubMed PMID: 25629663.
- Ewer K*, **Rampling T*,** Venkatraman N* *et al.* A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. N Engl J Med. 2016 Apr 28;374(17):1635-46. PMID: 25629663 *Equal contribution – joint first author. Follow up to preliminary report (above)

5.2. Introduction

In phase Ia, Dose-Escalating, Safety and Immunogenicity trial, I assessed the safety and immunogenicity in healthy UK adults of a monovalent chimpanzee adenovirus vectored vaccine expressing the Zaire strain Ebolavirus glycoprotein (ZEBOV GP), either alone or as part of a heterologous prime boost regimen with the multivalent MVA-BN® Filo.

5.3. Objectives

5.3.1. Primary Objective:

To assess the safety of a single dose of the novel monovalent Ebola Zaire candidate vaccine ChAd3-EBO Z at escalating doses, and of the heterologous prime boost regimen of ChAd3-EBO Z followed by MVA-BN® Filo 3-10 weeks later;

To assess the safety of the heterologous prime boost regimen of ChAd3-EBO Z 2.5 (± 1.2) x 10^{10} vp followed by MVA-BN® Filo 1-2 weeks later at doses of 2.2 x 10^{8} TCID50s (1.5 x 10^{8} pfu) and 4.4 x 10^{7} TCID50s (3 x 10^{7} pfu)

5.3.2. Secondary Objectives:

To assess immunogenicity of 3 different doses of the novel monovalent Ebola Zaire candidate vaccine, ChAd3-EBO Z, and of the heterologous prime boost regimen of ChAd3-EBO Z followed by MVA-BN® Filo 3-10 weeks later;

To assess the immunogenicity of the heterologous prime boost regimen of ChAd3-EBO Z 2.5 (± 1.2) x 10^{10} vp followed by MVA-BN® Filo 1-2 weeks later at doses of 2.2 x 10^{8} TCID50s (1.5 x 10^{8} pfu) and 4.4 x 10^{7} TCID50s (3 x 10^{7} pfu)

5.4. Participants

Recruitment and vaccination for this study was conducted at the Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, UK. Participants were healthy UK adults aged 18-50 years who provided written informed consent and the study was conducted according to the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice (GCP).

5.5. Ethical and Regulatory Approval

The study was reviewed and approved by the UK National Research Ethics Service (NRES), Committee South Central – Oxford A (OXREC A; Ref: 14/SC/1256), the Medicines and Healthcare products Regulatory Agency (MHRA; Ref: 21584/0334/001-0001) and the Oxford University Clinical Trials and Research Governance team, who monitored GCP compliance. The trial was registered with ClinicalTrials.gov (Ref: NCT02240875). Safety oversight was provided by an independent Data Safety Monitoring Board (DSMB).

5.6. Study Design

EBL01 was a Phase I dose-escalation open-label study to assess the safety and immunogenicity of the investigational monovalent Zaire strain Ebolavirus vaccine ChAd3-EBO Z and the multivalent filoviral vaccine MVA-BN® Filo in healthy adults. Eligible subjects were allocated to receive ChAd3-EBO Z as a single intramuscular (IM) injection at day 0 (D0) in one of three dose groups; Group 1 (1 x 10^{10} vp), Group 2 (2.5 x 10^{10} vp), Group 3 (5 x 10^{10} vp). The first 9 vaccinations occurred in a stepwise dose escalation manner, with 3 subjects in the lower dose group being vaccinated and followed up for a minimum of 48 hours before proceeding to the next dose group. In addition, of the subjects previously vaccinated with ChAd3-EBO Z, 30 subjects subsequently received a boosting vaccination with MVA-BN® Filo at one of 2 doses, 2.2 x 10^8 TCID50s (1.5 x 10^8 pfu) or 4.4 x 10^8 TCID50s (3 x 10^8 pfu). Four extension groups were subsequently added to assess the impact of a further

reduced prime boost interval of either 1 or 2 weeks, and also of a reduced boosting dose of MVA-BN® Filo. Further details of study conduct can be found in Chapter 2: Materials and Methods and in the appendix Table 47 to Table 50.

5.6.1. Study Groups

See Table 32 and Table 33

Group 1: 1 x 10^{10} vp administered IM by needle and syringe (n=20)

Group 2: 2.5 x 10^{10} vp administered IM by needle and syringe (n=20)

Group 3: 5 x 10^{10} vp administered IM by needle and syringe (n=20)

30 subjects (n=10 per prime dose group) were then allocated to one of 2 subgroups to receive vaccination with MVA-BN® Filo 3-10 weeks after ChAd3-EBO Z prime as follows:

Subgroup b: MVA-BN® Filo at 3 x 10^8 pfu (4.4 x 10^8 $TCID_{50s}$) administered IM by needle and syringe (n=12, n=4 per prime dose group)

Subgroup c: MVA-BN® Filo at 1.5 x 10^8 pfu (2.2 x 10^8 $TCID_{50s}$) administered IM by needle and syringe (n=18, n=6 per prime dose group)

Group	1		2		3	
Number	20		20		20	
Week 0 ChAd3-EBO Z Dose (vp)	1 x 10^{10}		2.5 x 10^{10}		5 x 10^{10}	
Group	1b	1c	2b	2c	3b	3c
Number	4-6	4-6	4-6	4-6	4-6	4-6
Week 3-10 MVA-BN® Filo Dose (TCID50s)	4.4 x 10^8	2.2 x 10^8	4.4 x 10^8	2.2 x 10^8	4.4 x 10^8	2.2 x 10^8

Table 32: EBL01 Study dose groups

An extension to the study added 4 further groups with a reduced prime boost interval.

Group 4: ChAd3-EBO Z at 2.5 x 10^{10} vp IM followed by MVA-BN® Filo at 1.5 x 10^{8} pfu (2.2 x 10^{8} $TCID_{50s}$) 1 week later (n=8).

Group 5: ChAd3-EBO Z at 2.5 x 10^{10} vp IM followed by MVA-BN® Filo at 1.5 x 10^{8} pfu (2.2 x 10^{8} $TCID_{50s}$) 2 weeks later (n=8)

Group 6: ChAd3-EBO Z at 2.5 x 10^{10} vp IM followed by MVA-BN® Filo at 3 x 10^{7} pfu (4.4 x 10^{7} $TCID_{50s}$) 1 week later (n=8).

Group 7: ChAd3-EBO Z at 2.5 x 10^{10} vp IM followed by MVA-BN® Filo at 3 x 10^{7} pfu (4.4 x 10^{7} $TCID_{50s}$) 2 weeks later (n=8)

Group	4	5	6	7
Number	8	8	8	8
Week 0 ChAd3-EBO Z Dose (vp)	2.5 (± 1.2) x 10^{10}	2.5 (± 1.2) x 10^{10}	2.5 (± 1.2) x 10^{10}	2.5 (± 1.2) x 10^{10}
Week 1 MVA-BN® Filo Dose (TCID50s)	2.2 x 10^{8} (1.5 x10^{8} pfu)	-	4.4 x 10^{7} (3 x10^{7} pfu)	-
Week 2 MVA-BN® Filo Dose (TCID50s)	-	2.2 x 10^{8} (1.5 x10^{8} pfu)	-	4.4 x 10^{7} (3 x10^{7} pfu)

Table 33: EBL01 Extension dose groups

5.6.1.44. Progression of dose escalation

The dose escalation process for ChAd3-EBO Z and MVA-BN® Filo is described in detail in section 2.4 and Figure 63.

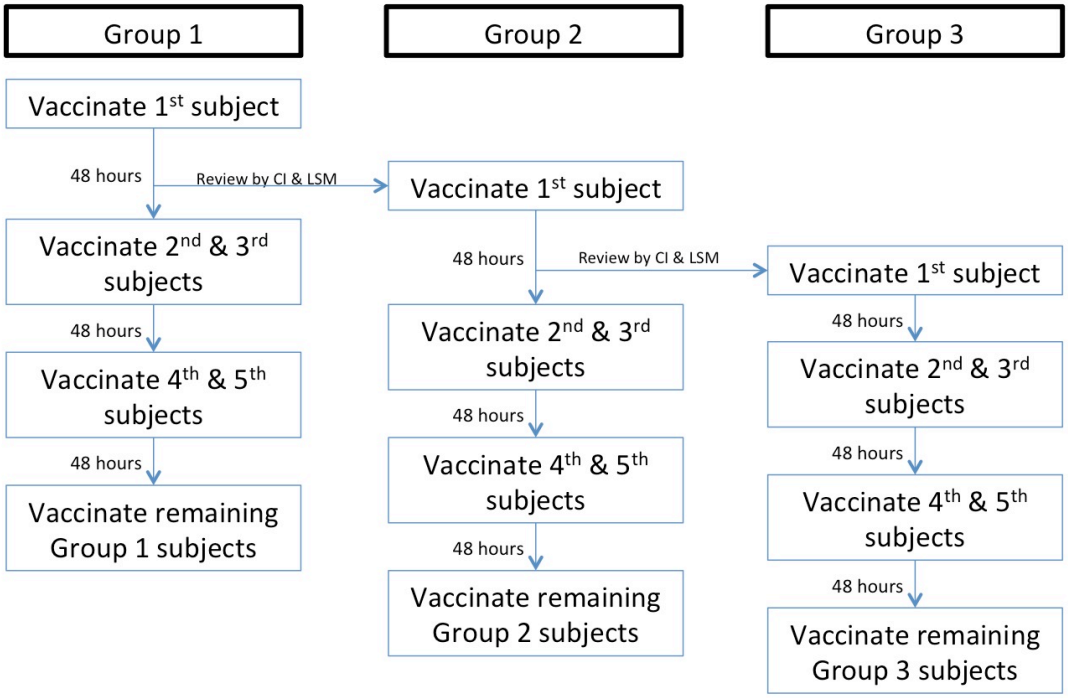


Figure 63: Pathway for dose escalation and vaccination of subjects in Groups 1, 2 and 3

All vaccinations were administered intramuscularly (IM) into the deltoid region of the arm.

Further details of the inclusion/exclusion criteria, the vaccines, clinical follow-up, safety monitoring, immunological and molecular methods, and statistics can be found in Chapter 2: Materials & Methods.

5.7. Results

5.7.1. Participants

Recruitment took place between September 2014 and March 2016. Ninety one healthy adult volunteers were enrolled. Demographics for the subjects enrolled are shown in Table 12. One hundred and forty-two subjects were screened for eligibility and 102 subjects were identified as eligible. Twenty subjects were allocated to each of Groups 1, 2 and 3 to receive ChAd3-EBO Z at one of three doses. Ten subjects from each dose group were subsequently invited to return for a booster dose of MVA-BN® Filo at either $3x10^8$ pfu (subgroups 1b, 2b and 3b) or $1.5x10^8$ pfu (subgroups 1c, 2c and 3c). Eight subjects were allocated to each of Groups 4, 5 and 6, and 7 subjects to Group 7 to receive ChAd3-EBO Z at $2.5x10^{10}$ vp and MVA-BN® Filo at either $1.5x10^8$ pfu (Groups 4 and 5) or $3x10^7$ pfu (Groups 6 and 7) either 1 or 2 weeks later. Vaccinations with ChAd3-EBO Z began on 17th Sept 2014, and were completed by 18th November 2014. Vaccinations with MVA-BN® Filo began on 26th November 2014 and were completed by 9th December 2014. Vaccinations in the extension groups (4-7) began on 10th Feb 2015, and were completed by 11th May 2016. A single subject withdrew from Group 1 after ChAd3 vaccination, citing personal reasons. This subject declined to return for any further follow up visits. There were no withdrawals due to safety concerns and no pre-defined study stopping or holding rules were activated. A flow diagram of recruitment can be found in Figure 64.

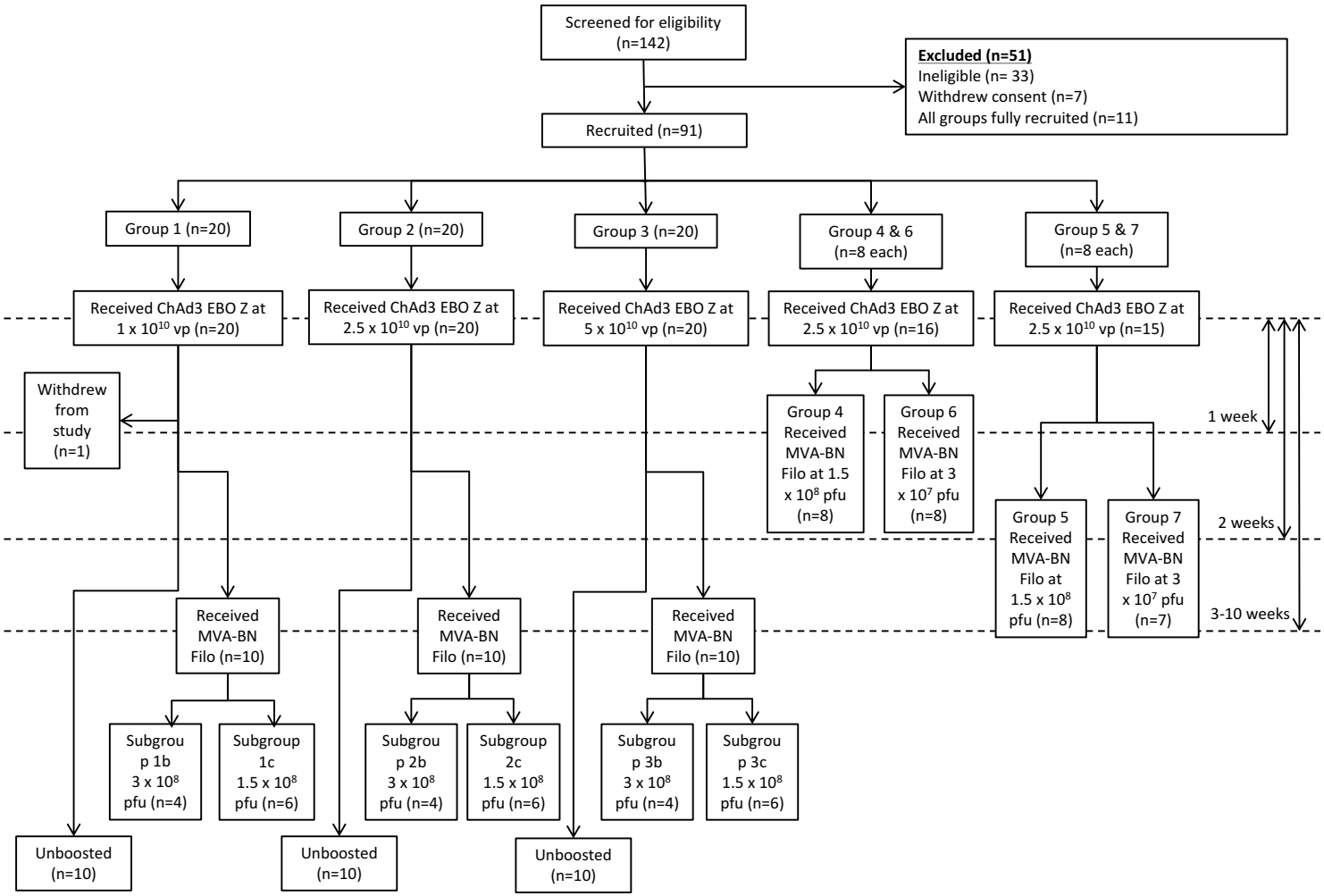


Figure 64: EBL01 flow chart of study design and volunteer recruitment

	Group 1		Group 2		Group 3						
	Unboosted (n = 10)	Boosted (1b & 1c) (n=10)	Unboosted (n = 10)	Boosted (1b & 1c) (n=10)	Unboosted (n = 10)	Boosted (1b & 1c) (n=10)	Group 4 n=8	Group 5 n=8	Group 6 n=8	Group 7 n=7	All Groups n (%)
Gender											
Male	1 (90)	5 (50)	8 (80)	5 (50)	7 (70)	6 (60)	3 (37.5)	4 (50)	2 (25)	2 (28.6)	43 (47.3)
Female	9 (10)	5 (50)	2 (20)	5 (50)	3 (30)	4 (40)	5 (62.5)	4 (50)	6 (75)	5 (71.4)	48 (52.7)
Age											
18-20	0	0	0	0	3 (30)	0	0	1 (12.5)	1 (12.5)	1 (14.3)	6 (6.6)
21-30	4 (40)	5 (50)	4 (40)	6 (60)	2 (20)	7 (70)	6 (75)	5 (62.5)	6 (75)	6 (85.7)	51 (56)
31-40	4 (40)	3 (30)	3 (30)	3 (30)	2 (20)	1 (10	1 (12.5)	1 (12.5)	1 (12.5)	0	19 (20.9)
>41	2 (20)	2 (20)	3 (30)	1 (10)	3 (30)	2 (20)	1 (12.5)	1 (12.5)	0	0	15 (16.5)
Mean (+/- SD)	32.2±10.0	30.4±8.2	35.2±8.2	30.8±7.4	31.9±11.2	39.9±7.6	28.0±10.0	27.8±8.1	25.1±5.3	23.1±2.7	29.8±8.6
Range	21–48	22-42	24–48	22-48	18–48	22-42	21-50	20-43	20-36	20-27	18–50
Ethnicity											
White	9 (90)	10 (100)	10 (100)	9 (90)	9 (90)	9 (90)	7 (87.5)	7 (87.5)	7 (87.5)	6 (85.7)	83 (91.2)
Black	0	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	1 (10)	1 (10)	0	1 (12.5)	1 (12.5)	1 (12.5)	0	5 (5.5)
Mixed	1 (10)	0	0	0	0	1 (10)	0	0	0	1 (14.3)	3 (3.3)
BMI											
<18.5	0	0	1 (10)	0	0	1 (10)	0	0	0	0	2 (2.2)
18.5-24.9	6 (60)	5 (50)	5 (50)	6 (60)	8 (80)	8 (80)	6 (75)	6 (75)	6 (75)	6 (85.7)	61 (67)
25-29.9	3 (30)	5 (50)	3 (30)	3 (30)	2 (20)	1 (10)	2 (25)	2 (25)	1 (12.5)	1 (14.3)	24 (26.4)
>30	1 (10)	0	1 (10)	1 (10)	0	0	0	0	1 (12.5)	0	4 (4.4)
Mean (+/- SD)	23.8±4.3	25.2±1.9	24.5±4.0	24.7±2.9	22.7±2.0	22.8±2.7	22.2±3.7	23.4±3.1	24.6±5.6	22.2±2.4	23.7±3.4
Range	18.5–30.5	22.0-28.5	17.4–33.0	21.4-30.7	20.4–26.6	17.1-27.4	18.6-27.8	18.5-29.0	19.8-37	19.4-26.3	17.1–37.0

Table 34: Demographics of all subjects enrolled in EBL01

5.7.2. Safety

5.7.2.45. Serious Adverse Events (SAEs)

In this trial, there have been 2 SAEs and no SUSARs. Neither SAE was deemed related to vaccination, and each is described in detail below. No pregnancies or deaths have occurred.

SAE #1

This subject returned from Sierra Leone, where he had been working, in February 2016. Following his return, he had felt unwell and was admitted to Salisbury hospital on the 23rd of March 2016.

He was subsequently referred to Southampton's infectious disease clinic where an eventual diagnosis of schistosomiasis was made, and he was treated successfully with praziquantel. Treatment was completed on the 8th June 2016.

This patient had received ChAd3-EBO Z at $2.5x10^{10}$vp on 14th Oct 2014 and MVA-BN® Filo at $3x10^{8}$pfu on 3rd December 2014. This SAE was assessed as being unrelated to vaccination.

SAE #2

This subject had onset of weakness in both legs secondary to a herniated intervertebral disc. This was diagnosed by MRI and the patient underwent emergency discectomy and nerve root compression on the 7th February 2017. The

patient was discharged from orthopaedic follow up on 20th March 2017. This patient had received ChAd3-EBO Z at 2.5 x 10^{10}vp on 11th Nov 2014 and MVA-BN® Filo at 1.5 x 10^{8}pfu on 3rd December 2014. This SAE was assessed as being unrelated to vaccination.

5.7.2.46. Adverse events following ChAd3-EBO Z

The majority of solicited adverse events (AEs) following each vaccination with ChAd3-EBO Z were mild in severity and all resolved. Commonly reported adverse events were vaccine site pain (70% of subjects), warmth (34%), feverishness (24%), myalgia (42%), fatigue (54%), headache (56%) and malaise (40%) (Table 35, Figure 65). There were single reports each of severe nausea, myalgia and malaise, all in intermediate (2.5 x 10^{10}vp) dose group subjects. All severe solicited AEs were short lived and spontaneously resolved.

Unsolicited AEs were collected until day 28 after vaccination, or until vaccination with MVA-BN® Filo (whichever came first), at which point AEs were collected for a further 28 days on a separate diary card. Verbatim reports of unsolicited signs and symptoms were reviewed by a physician and coded and tabulated according to the MedDRA Dictionary for Adverse Reaction Terminology (See Table 69 to Table 72 in the appendix for full listings of unsolicited AEs). Each unsolicited AE was annotated with a causality grading according to likely relationship to vaccination of either Unrelated, Unlikely, Possible, Probable or Definite. The numbers of subjects with at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported up to 28 days (Day 0-Day 27) after vaccination (or until vaccination with MVA-BN® Filo) have been tabulated and are shown in Table

36. The majority of unsolicited adverse events were mild in severity and resolved spontaneously. There was a single subject in the intermediate dose group who reported a severe headache at day 9 after vaccination that was deemed possibly related. This persisted at grade 3 severity for ≤24 hours only, and had completely resolved by day 13.

Prolonged aPTT was observed in 4 subjects occurring within the first two weeks following vaccination with ChAd3-EBO Z in 3 group 2 subjects (2 x AE grade 1 and 1 x AE grade 2 elevations) and a grade 1 elevation in a single high dose group subject. None of these aPTT prolongations were associated with symptoms or clinical features of coagulopathy. The aPTT elevation fully resolved in all subjects. No further abnormality was found in any of these subjects on extended laboratory haematological and coagulation evaluation. Transiently induced antiphospholipid antibodies have previously been reported following administration of adenovirus vectors leading to an in vitro artifact on the laboratory assay for aPTT. [172-174] Transient mild lymphopenia was noted on day 1 following vaccination with ChAd3-EBO Z in 5 low dose group subjects, 5 intermediate dose group 2 subjects and 8 group 3 subjects, and moderate lymphopenia was noted in 2 subjects on day 1 in both groups 2 and 3. Transient mild to moderate elevations in bilirubin were recorded in 6 subjects in groups 2 and 3 only. Transient hyperbilirubinaemia in the severe range was recorded in 2 subjects (1 in each of groups 2 and 3), both with a pre-vaccination diagnosis of Gilbert's syndrome. A full tabulation of all laboratory adverse events recorded after vaccination with ChAd3-EBO Z can be found in Table 39.

5.7.2.47. Adverse events following MVA-BN® Filo

As with ChAd3-EBO Z, the majority of solicited AEs following each vaccination with MVA-BN® Filo were transient, spontaneously resolving and mild in severity. There was a higher reported frequency of vaccine site pain, especially in the intermediate (94% of subjects) and (high 92%) dose groups. Grade 3 pain was reported by a single subject (1.9%) only in the intermediate dose group, and mild pain only was reported in the low dose groups (53%). Other commonly reported AEs were myalgia (38%), fatigue (41%), headache (39%) and malaise (26%) (Table 37, Figure 66). There was a dose related effect on AE frequency following vaccination with MVA-BN® Filo, with a higher proportion of all solicited adverse events in the high dose group when compared with the low and intermediate dose groups. Conversely, there were no grade 3 solicited AEs in the high dose group, but isolated subject reports of grade 3 pain, swelling, nausea or malaise in either the low or intermediate dose groups.

Unsolicited AEs were collected until day 28 after vaccination. Reports of unsolicited signs and symptoms were reviewed, coded and annotated with a causality grading as per unsolicited AEs after ChAd3-EBO Z. The numbers of subjects with at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported up to 28 days (Day 0-Day 27) after vaccination have been tabulated and are shown in Table 38. The majority of subjects (67%) either reported mild, or no unsolicited AEs, with 28% reporting moderate AEs and 5% reporting severe AEs. There was a single subject in the intermediate dose group who reported severe vomiting at day 7 after vaccination that was deemed possibly related. This AE

persisted for ≤24 hours only. Full listings of all unsolicited AEs can be found in the appendix Table 69 to Table 72.

There was a low frequency of transient laboratory AEs after vaccination with MVA-BN® Filo. A full tabulation of all laboratory AEs can be found in Table 40. Severe hyperbilirubinaemia was again detected in a single subject with a pre-vaccination diagnosis of Gilbert's disease. In this subject serum bilirubin was in the moderate AE range prior to vaccination. There was a lower incidence of laboratory AEs in the low dose groups when compared with the intermediate and high dose groups.

Symptom/Intensity	Group 1 (n=19)	Groups 2, 4-7 (n=51)	Group 3 (n=20)	Total (n=90)
Local	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Pain				
Mild	10 (52.6)	39 (76.5)	7 (35)	56 (62.2)
Moderate	2 (10.5)	2 (3.9)	3 (15)	7 (7.8)
Mild swelling	3 (15.8)	2 (3.9)	2 (10)	7 (7.8)
Mild redness	3 (15.8)	9 (17.6)	3 (15)	15 (16.7)
Warmth				
Mild	10 (52.6)	9 (17.6)	4 (20)	23 (25.6)
Moderate	0	1 (2.0)	0	1 (1.1)
Mild itch	2 (10.5)	5 (9.8)	0	7 (7.8)
Systemic				
Fever				
Mild	0	1 (2.0)	0	1 (1.1)
Moderate	0	1 (2.0)	1 (5)	2 (2.2)
Feverishness				
Mild	3 (15.8)	7 (13.7)	6 (30)	16 (17.8)
Moderate	0	4 (7.8)	1 (5)	5 (5.6)
Severe	0	1 (2.0)	0	1 (1.1)
Myalgia				
Mild	7 (36.8)	14 (27.5)	11 (55)	32 (35.6)
Moderate	0	5 (9.8)	0	5 (5.6)
Severe	0	1 (2.0)	0	1 (1.1)
Arthralgia				
Mild	1 (5.3)	8 (15.7)	3 (15)	12 (13.3)
Moderate	0	4 (7.8)	1 (5)	5 (5.6)
Headache				
Mild	7 (36.8)	28 (54.9)	8 (40)	43 (47.8)
Moderate	2 (10.5)	4 (7.8)	1 (5)	7 (7.8)
Fatigue				
Mild	11 (57.9)	18 (35.3)	10 (50)	39 (43.3)
Moderate	2 (10.5)	5 (9.8)	3 (15)	10 (11.1)
Nausea				
Mild	3 (15.8)	5 (9.8)	4 (20)	12 (13.3)
Moderate	1 (5.3)	2 (3.9)	1 (5)	4 (4.4)
Severe	0	1 (2.0)	0	1 (1.1)
Malaise				
Mild	6 (31.6)	15 (29.4)	7 (35)	28 (31.1)
Moderate	2 (10.5)	3 (5.9)	2 (10)	7 (7.8)
Severe	0	1 (2.0)	0	1 (1.1)

Table 35: Table of occurrence of solicited adverse events reported in the 7 day period after vaccination with ChAd3-EBO Z. Each AE is counted once only at maximum severity reported in each subject.

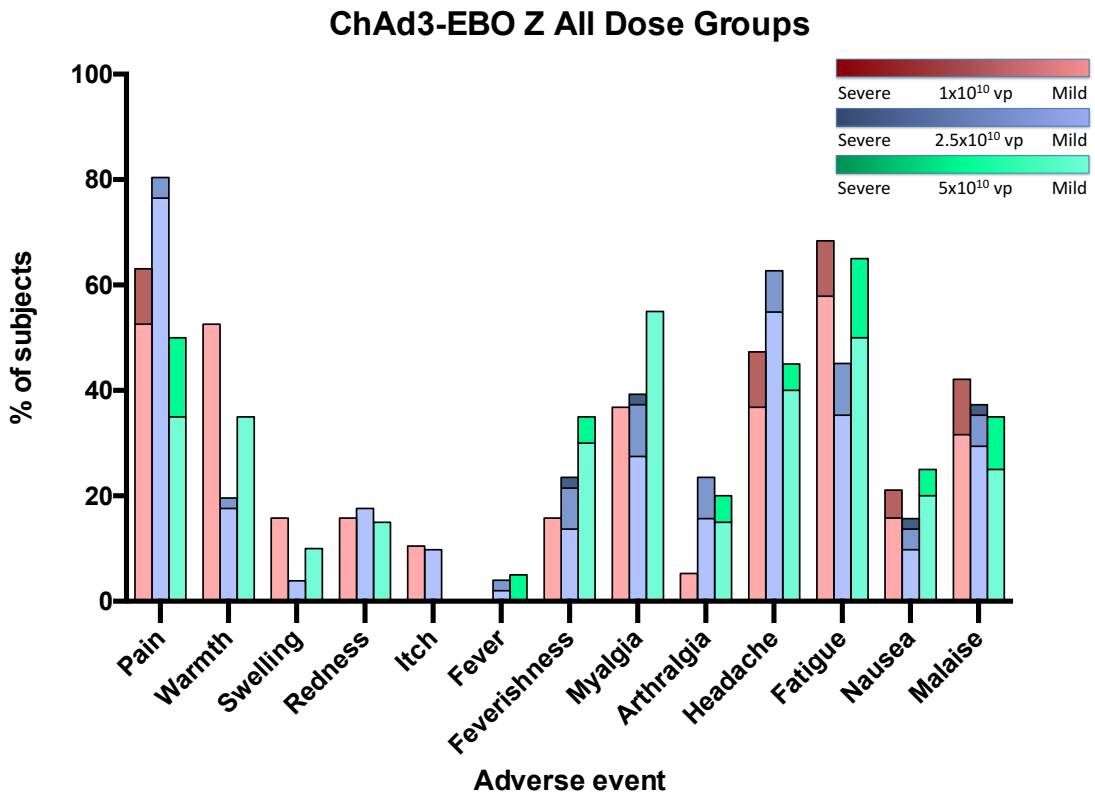


Figure 65: Solicited reactogenicity in the 7-day period following vaccination with ChAd3-EBO Z at 1x10^{10} vp (Group 1), 2.5x10^{10} vp (Groups 2, 4, 5, 6 & 7) or 5x10^{10} vp (Group 3). Increasing AE severity (mild, moderate or severe) is represented by darker shading. Each AE is recorded once at maximum severity for each subject.

	Group 1 (n=19)	Groups 2, 4, 5, 6 & 7 (n=51)	Group 3 (n=20)	All Groups (n=90)
All unsolicited AEs	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Mild	14 (73.7)	17 (33.3)	9 (45.0%)	40 (44.4)
Moderate	2 (10.5)	9 (17.6)	8 (40.0%)	19 (21.1)
Severe	0	2 (3.9)	0	2 (2.2)
Unsolicited AEs deemed possibly, probably or definitely related to vaccination				
Mild	7 (36.8)	16 (31.4)	9 (45.0%)	32 (35.6)
Moderate	2 (10.5)	5 (9.8)	3 (15.0%)	10 (11.1)
Severe	0	1 (2.0)	0	1 (1.1)

Table 36: Maximum unsolicited reactogenicity summary after vaccination with ChAd3-EBO Z at 1x10^{10} vp (Group 1), 2.5x10^{10} vp (Groups 2, 4, 5, 6 & 7) or 5x10^{10} vp (Group 3. Unsolicited reactogenicity is collected to day 28 after vaccination, or until receipt of MVA-BN® Filo vaccination. Causality has been assigned to all

unsolicited AEs based on type of event and temporal relationship to vaccination. This process is further described in Chapter 2. Causality gradings are: unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination.

Symptom/Intensity	Subgroup b (3×10^8 pfu)	Subgroup c & Groups 4 & 5 (1.5×10^8 pfu)	Groups 6 & 7 (3×10^7 pfu)	All Groups
Local AEs	**n=12**	**n=34**	**n=15**	**n=61**
Pain	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Mild	5 (41.7)	26 (76.5)	8 (53.3)	39 (63.9)
Moderate	6 (50.0)	5 (14.7)	0	11 (18)
Severe	0	1 (2.9)	0	1 (1.6)
Swelling				
Mild	3 (25.0)	10 (29.4)	0	13 (21.3)
Severe	0	1 (2.9)	0	1 (1.6)
Redness				
Mild	7 (58.3)	8 (23.5)	4 (26.7)	19 (31.1)
Warmth				
Mild	4 (33.3)	10 (29.4)	2 (13.3)	16 (26.2)
Moderate	1 (8.3)	2 (5.9)	0	3 (4.9)
Itch				
Mild	2 (16.7)	1 (2.9)	1 (6.7)	4 (6.6)
Systemic AEs				
Fever				
Mild	3 (25.0)	1 (2.9)	0	4 (6.6)
Feverishness				
Mild	1 (8.3)	1 (2.9)	1 (6.7)	3 (4.9)
Moderate	2 (16.7)	2 (5.9)	0	4 (6.6)
Myalgia				
Mild	5 (41.7)	9 (26.5)	3 (20)	17 (27.9)
Moderate	3 (25.0)	3 (8.8)	0	6 (9.8)
Arthralgia				
Mild	1 (8.3)	2 (5.9)	0	3 (4.9)
Moderate	1 (8.3)	1 (2.9)	0	2 (3.3)
Headache				
Mild	5 (41.7)	12 (35.3)	1 (6.7)	18 (29.5)
Moderate	2 (16.7)	2 (5.9)	2 (13.3)	6 (9.8)
Fatigue				
Mild	7 (58.3)	11 (32.4)	2 (13.3)	20 (32.8)
Moderate	1 (8.3)	3 (8.8)	1 (6.7)	5 (8.2)
Nausea				
Mild	1 (8.3)	2 (5.9)	1 (6.7)	4 (6.6)
Moderate	1 (8.3)	0	0	1 (1.6)

Severe	0	0	1 (6.7)	1 (1.6)
Malaise				
Mild	4 (33.3)	6 (17.6)	0	10 (16.4)
Moderate	2 (16.7)	2 (5.9)	1 (6.7)	5 (8.2)
Severe	0	0	1 (6.7)	1 (1.6)

Table 37: Table of occurrence of solicited adverse events reported in the 7 day period after vaccination with MVA-BN® Filo. Each AE is counted once only at maximum severity reported in each subject.

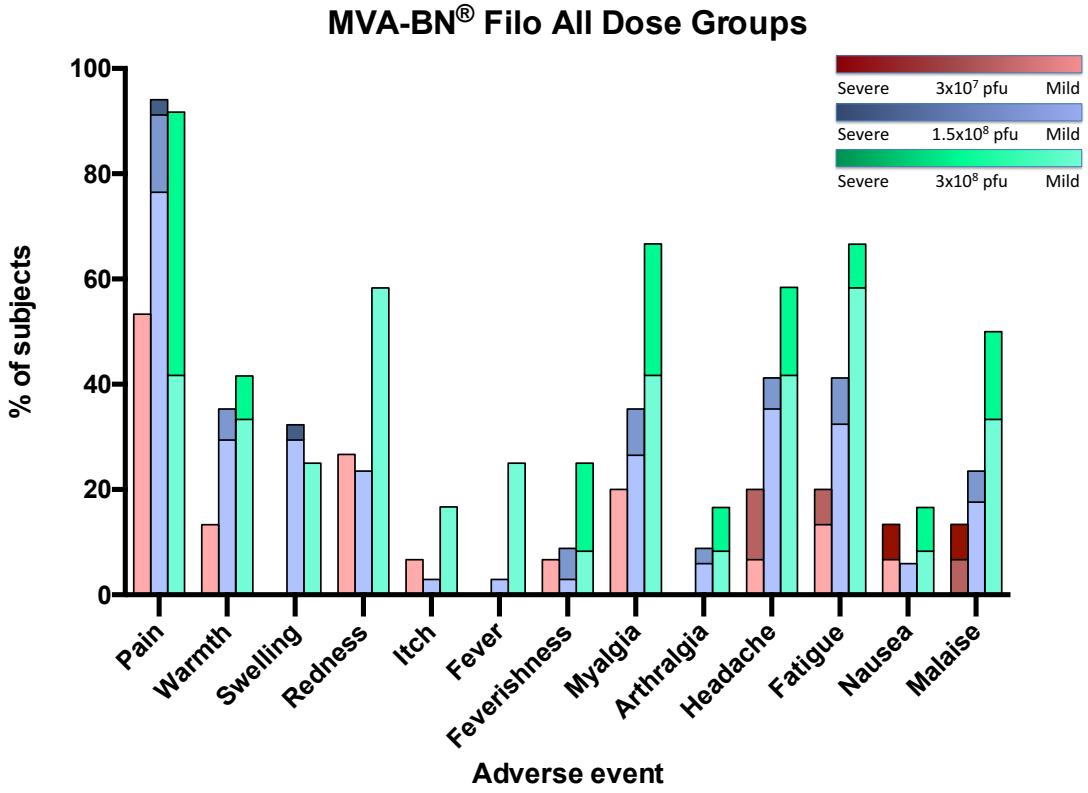


Figure 66: Solicited reactogenicity in the 7-day period following vaccination with MVA-BN® Filo at 3x10⁷ pfu (Groups 6 & 7), 1.5x10⁸ pfu (Groups 1c, 2c, 3c, 4 & 5) or 3x10⁸ pfu (Group 1b, 2b & 3b). Increasing AE severity (mild, moderate or severe) is represented by darker shading. Each AE is recorded once at maximum severity for each subject

	Subgroup b (n=12)	Subgroup c & Groups 4 & 5 (n=34)	Groups 6 & 7 (n=15)	All Groups (n=61)
All unsolicited AEs	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Mild	3 (25.0)	12 (35.3)	1 (6.7)	16 (26.2)
Moderate	5 (41.7)	9 (26.5)	3 (20)	17 (27.9)
Severe	0	2 (5.9)	1 (6.7)	3 (4.9)
Unsolicited AEs deemed possibly, probably or definitely related to vaccination				
Mild	5 (41.7)	14 (41.2)	1 (6.7)	20 (32.8)
Moderate	2 (16.7)	4 (11.8)	3 (20)	9 (14.8)
Severe	0	1 (2.9)	0	1 (1.6)

Table 38: Maximum unsolicited reactogenicity summary after vaccination with MVA-BN® Filo at $3x10^8$ pfu (Subgroup b), 1.5. Unsolicited reactogenicity is collected to day 28 after vaccination. Causality has been assigned to all unsolicited AEs based on type of event and temporal relationship to vaccination. This process is further described in the stydy protocol. Causality gradings are: unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination.

Dose	$1x10^{10}$ vp	2.5×10^{10} vp	5×10^{10} vp	
	Group 1	Groups 2, 4, 5, 6 & 7	Group 3	Total
Lab AE/Severity	**(n=19)**	**(n=51)**	**(n=20)**	**(n=90)**
Anaemia				
Mild	6 (31.6)	1 (2)	0	7 (7.8)
Decreased fibrinogen				
Moderate	0	0	1 (5)	1 (1.1)
Thrombocytopenia				
Moderate	0	0	1 (5)	1 (1.1)
Elevated ALT				
Mild	1 (5.3)	0	0	1 (1.1)
Elevated aPTT				
Mild	0	2 (3.9)	1 (5)	3 (3.3)
Moderate	0	1 (2)	0	1 (1.1)
Eosinophilia				
Mild	0	0	2 (10)	2 (2.2)
Moderate	0	0	1 (5)	1 (1.1)
Hyperbilirubinaemia				
Mild	0	2 (3.9)	0	2 (2.2)
Moderate	0	1 (2)	3 (15)	4 (4.4)
Severe	0	1‡ (2)	1 (5)	2 (2.2)
Hypernatraemia				
Mild	1 (5.3)	1 (2)	0	2 (2.2)
Hypokalaemia				
Mild	0	2 (3.9)	1 (5)	3 (3.3)
Hyponatraemia				
Mild	1 (5.3)	1 (2)	0	2 (2.2)
Moderate	1 (5.3)	0	0	1 (1.1)
Lymphopenia				
Mild	5 (26.3)	5 (9.8)	8 (40)	18 (20)
Moderate	0	2 (3.9)	2 (10)	4 (4.4)
Neutropenia				
Mild	1 (5.3)	1 (2)	2 (10)	4 (4.4)

Table 39: All laboratory adverse events following ChAd3-EBO Z. Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0. This table shows results for 75 volunteers followed to trial end, or until boosting vaccination with MVA BN®-Filo vaccination, whichever came first. ‡Severe hyperbilirubinaemia detected in a single subject with a pre-vaccination diagnosis of Gilbert's disease. Serum bilirubin was in the moderate AE range before vaccination, in this subject.

Lab AE/Severity	Subgroup b (n=12)	Subgroup c, Groups 4 & 5 (n=34)	Groups 6 & 7 (n=15)	Total (n=61)
Anaemia	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Mild	1 (8.3)	0	2 (13.3)	4 (6.6)
Moderate	0	1 (2.9)	1 (6.7)*	2 (3.2)
Hyperbilirubinaemia				
Mild	1 (8.3)	1 (2.9)	0	2 (3.2)
Moderate	0	2 (5.9)	0	2 (3.2)
Severe	0	1 (2.9)‡	0	1 (1.6)‡
Hypokalaemia				
Mild	4 (33.3)	1 (2.9)	0	5 (8.2)
Moderate	0	1 (2.9)	0	1 (1.6)
Hyponatraemia				
Mild	0	2 (5.9)	0	2 (3.2)
Lymphopenia				
Mild	4 (33.3)	6 (17.6)	0	10 (16.4)
Moderate	2 (16.7)	2 (5.9)	0	4 (6.6)
Neutropenia				
Mild	0	1 (2.9)	0	1 (1.6)
Raised ALT				
Mild	1 (8.3)	0	0	1 (1.6)

Table 40: All laboratory adverse events following MVA-BN® Filo. Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0. This table shows results for 59 subjects followed to at least day 28. ‡Severe hyperbilirubinaemia detected in a single subject with a pre-vaccination diagnosis of Gilbert's disease. Serum bilirubin was in the moderate AE range before vaccination, in this subject. *Moderate anaemia detected in a single subject with a pre-vaccination haemoglobin in the mild anaemia range.

5.7.3. Immunogenicity

5.7.3.48. Humoral response to vaccination

IgG responses to the Ebola Zaire glycoprotein were assessed in Groups 1-3 prior to immunisation and at days 14 and 28 (Figure 67, Figure 68). Responses peaked at 28

days post vaccination and there was no significant difference in response at any time point between dose groups. Vaccination induced a significant increase in titre compared with pre-vaccination titres in all dose groups, $p<0.0001$ (2-tailed Wilcoxon matched pairs test). Breadth of antibody titres post-ChAd3 prime was wide, with a high degree of variability between subjects. Comparison of responses at Day 14 and Day 28 post-prime between groups based on gender ($p=0.467$, $p=0.241$ and $p=0.877$ at Day 14 in Groups 1, 2 and 3 respectively; $p=0.244$, $p=0.351$ and $p=0.351$ at Day 28 in Groups 1, 2, and 3 respectively, Mann-Whitney test), ethnicity (white European vs other; $p=0.343$, $p=0.765$ and $p=0.353$ at Day 14 in Groups 1, 2 and 3 respectively; $p=0.087$, $p=0.616$ and $p=0.602$ at Day 28 in Groups 1, 2, and 3 respectively, Mann-Whitney test), age at enrolment (Spearman's $r=0.053$, $r=0.030$ and $r=0.209$ at Day 14 in Groups 1, 2 and 3 respectively; $r=0.024$, $r=0.057$ and $r=0.134$ at Day 28 in Groups 1, 2, and 3 respectively, $p>0.05$ for all comparisons), and BMI (Spearman's $r=0.132$, $r=0.162$ and $r=0.120$ at Day 14 in Groups 1, 2 and 3 respectively; $r=0.007$, $r=0.074$ and $r=0.247$ at Day 28 in Groups 1, 2, and 3 respectively, $p>0.05$ for all comparisons), at baseline did not reveal any significant associations. Although this variability was also observed following MVA in those subjects who received it, 100% of boosted subjects were positive for anti-GP Abs by day 14 after boosting (M+14). In subjects from groups 1-3 who were boosted with MVA-BN® Filo, antibody responses increased significantly by 7 days post-MVA and peaked at day 14 after boosting, dropping slightly by day 28 ($p<0.01$, Friedman test, $n=30$, Figure 69). Responses remained significantly above pre-boost levels at 180 days after MVA vaccination and were significantly higher than titres measured at 180 days after priming with ChAd3-EBO Z alone ($p=0.0101$, Mann-Whitney test, Figure 70). In pre-clinical macaque

efficacy studies using a lethal Ebolavirus challenge, and in a human Phase 1 trial of a bivalent ChAd3 vaccine, both conducted by NIH, an ELISA using an end-point titration method (EC_{90}) was described.[148,152] Pre- and post-ChAd3 vaccination samples were analysed using the NIH EC_{90} method in order to allow for direct comparison with protective antibody levels in the macaque efficacy trials. In the analyses of samples obtained from 58 participants on day 28, the geometric mean titre after the subtraction of prevaccination responses was 235 units in group 1, 402 in group 2, and 469 in group 3 (Figure 71). Using the NIH ELISA to determine antibody titres after boosting, the response peaked at fourteen days post MVA, with a GM titre of 11970 (95% confidence intervals [95%CI] 8762-16353), nearly twenty times higher than the peak level detected after priming with ChAd3, and well above the level identified as a correlate of vaccine efficacy in cynomolgus macaques[152] (Figure 72). Seven days after boosting, 90% of recipients had reciprocal titres ≥1000, a level associated with 77.1% efficacy in NHPs[152], this response rate increased to 100% at day 14 and was maintained at day 28.

Neutralising titres to the live Zaire Ebolavirus (Mayinga strain), from all boosted individuals were measured at day 28 post-ChAd3 and day 14 post-MVA (Figure 73). Low levels of neutralising antibodies were detected in subjects at day 28 (GM titre 14.9, 95%CI 12-18.5, very similar to levels reported after a recombinant VSV vaccine),[174] and these increased significantly by 9-fold to a GM titre of 139 (95%CI 90-215; $P<0.001$, 2-tailed Mann-Whitney test) with all subjects seropositive (titre>8) by day 14 post-MVA. Boosting with the higher dose of MVA elicited slightly higher neutralising antibody titres than the low dose (GMT in the high-dose group 243.9;

95% CI, 96 to 628; GMT in the low-dose group, 95.7; 95% CI, 65 to 142; P=0.03 by the two-tailed Mann–Whitney test).

Antibodies to the Sudan Ebolavirus glycoprotein were measured, but were not detected. This was to be expected in the absence of priming with this antigen, and suggests a lack of cross-reactivity between antibodies against the Sudan Ebolavirus GP and the Zaire Ebolavirus GP. However after boosting with MVA-BN® Filo, which expresses the Sudan Ebolavirus GP, IgG titres increased significantly (Figure 74, pre-vaccination GM titres 0.1, 95%CI 0.07-0.2, M+14 GMT 1.5, 95%CI 1.1-2.0, p<0.0001, Friedman test).

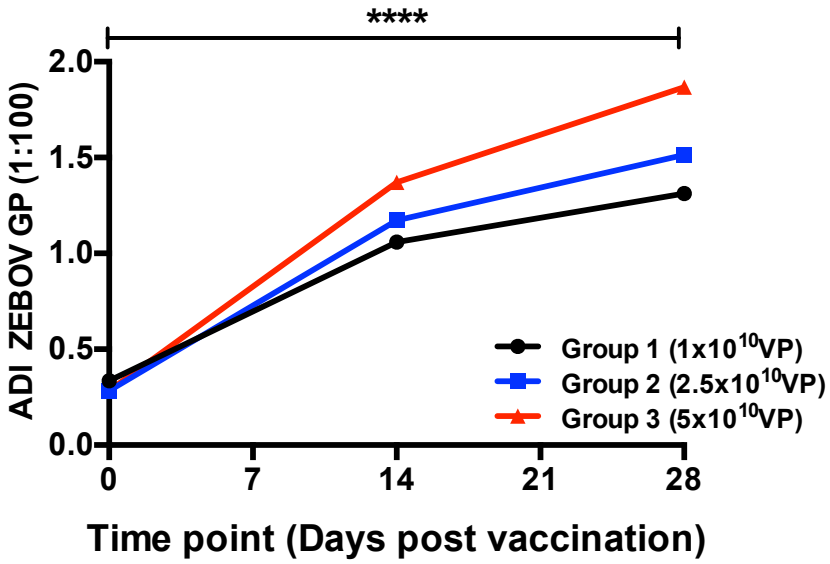


Figure 67: Anti-glycoprotein IgG levels measured with the ADI ELISA assay in Groups 1-3 at day 0 and days 14 and 28 days post ChAd3-EBO Z vaccination with increasing doses of vaccine. The Y axis units are of optical density (OD) without subtraction of background.

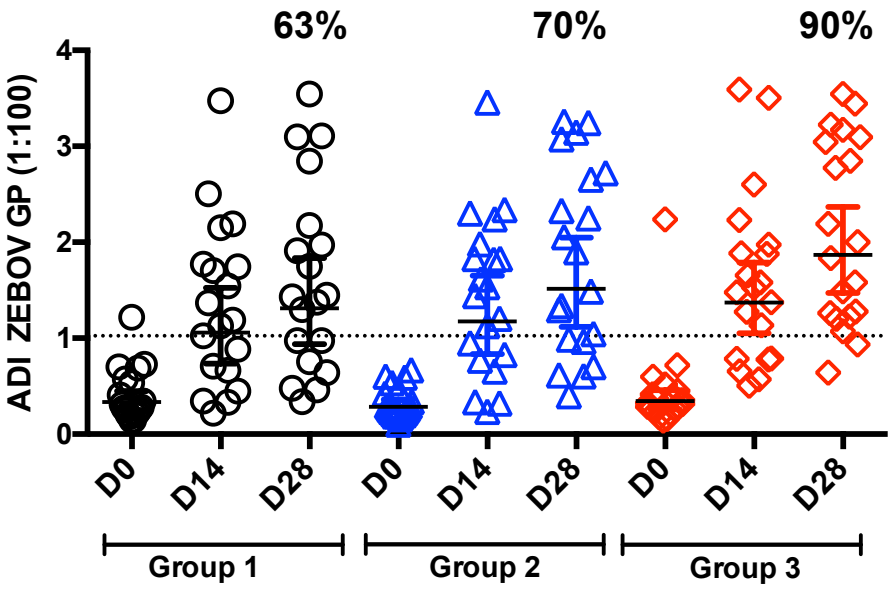


Figure 68: Individual responses post ChAd 3 EBO Z vaccination at days 0, 14, and 28 for Groups 1-3 in geometric means and 95% confidence intervals. The percentage of participants with positive results at the peak time points on day 28 are indicated. The dotted line represents the positive threshold (optical density, 1.024), as calculated from the mean +3 SD of the day 0 responses for all participants

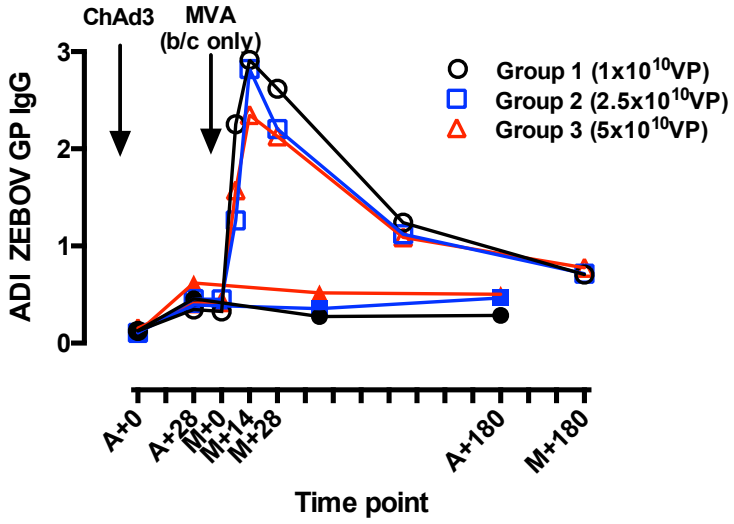


Figure 69: Geometric mean titres of antibody responses to increasing doses of the ChAd3-EBO Z either alone (solid symbols) or followed by a booster dose of MVA-BN® Filo (hollow symbols). Antibody responses are shown according to measurements on a standardized ELISA for Group 1 (n=19), Group 2 (n=20) and Group 3 (n=20). Timepoint after vaccination is represented on the x-axis by A+x for ChAd3 and M+x for MVA (where x=number of days)

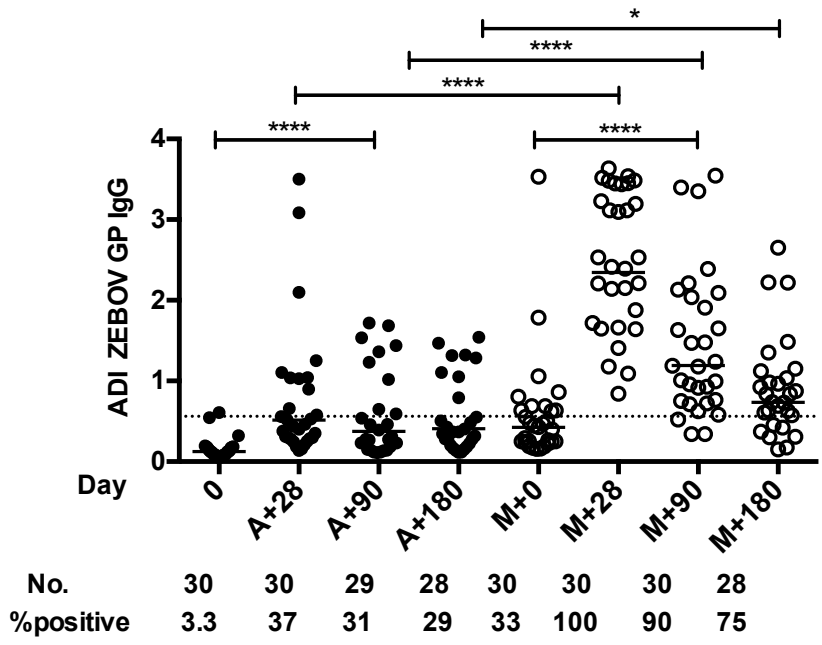


Figure 70: Responses as measured by anti-ZEBOV glycoprotein IgG ELISA after administration of the prime and boost vaccines. Solid horizontal lines represent the geometric mean titre. Percentages of subjects with positive antibody responses at each time point are indicated below the graph. The horizontal dashed line represents the threshold for a positive result (arbitrary ELISA units, +0.561), calculated as the mean plus 3 standard deviations of the response on day 0 for all participants.

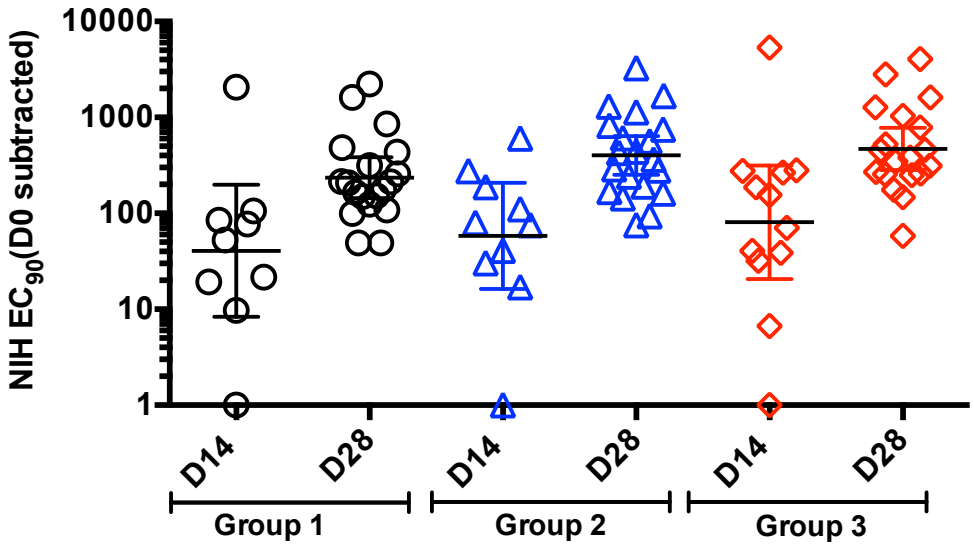


Figure 71: NIH EC90 ELISA titres following ChAd3 vaccination. The geometric mean titres in these groups were 235, 402, and 469, respectively, after the subtraction of prevaccination responses at baseline.

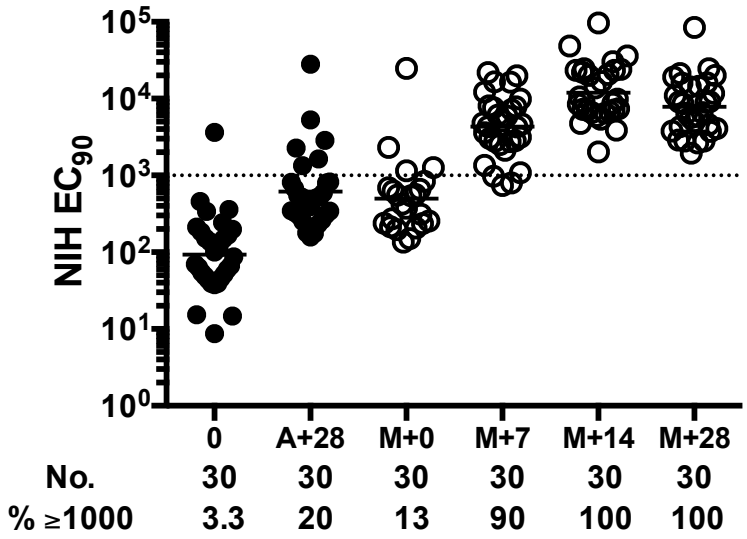


Figure 72: Reciprocal antibody titres measured using the NIH EC90 ELISA. Bars represent GMT. Percentage of vaccinees with titres ≥1000 (associated with 77% protection in macaques) are indicated. Dotted line represents a reciprocal titre of 1000.

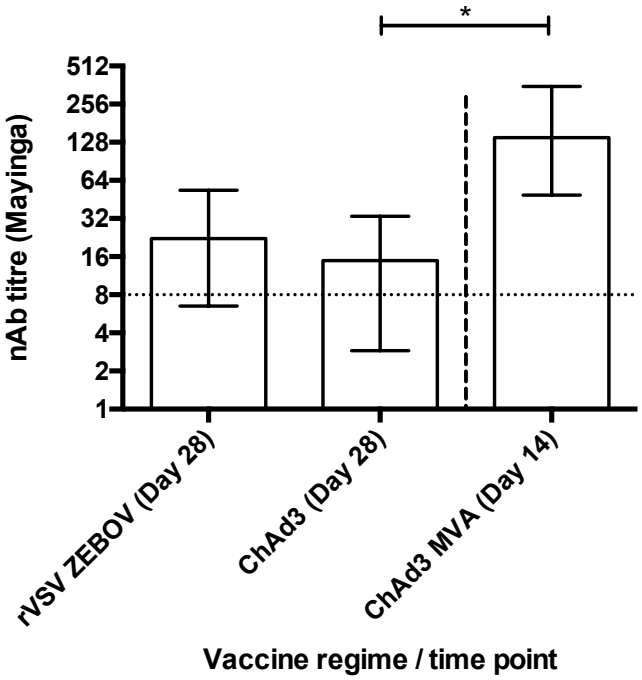


Figure 73: Neutralising antibody titres against live Zaire Ebolavirus (Mayinga strain) from all subjects boosted with MVA, measured at 28 days after the ChAd3 vaccination and 14 days after the MVA. Nab titres levels reported 28 days after the administration of rVSV-ZEBOV vaccine are shown for comparison.[174] By 14 days after MVA vaccination, the levels had increased by a factor of 9. Columns represent the geometric mean titre,

bars represent 95% confidence intervals, and the horizontal dashed lines represent the positive threshold. *=*P*<0.001 by the two-tailed Mann–Whitney test.

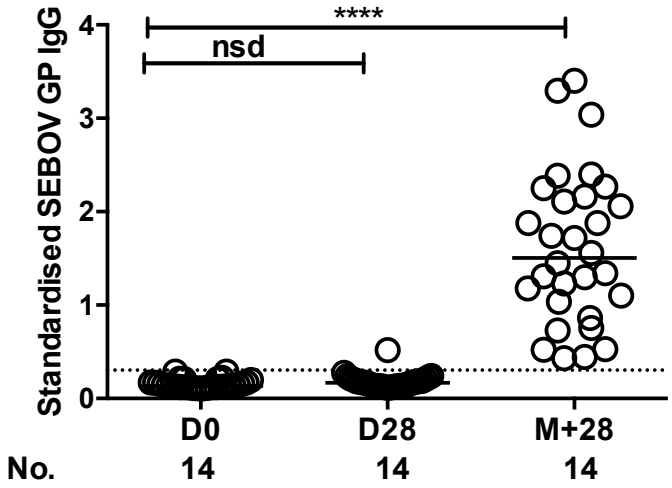


Figure 74: Antibody responses to Sudan virus glycoprotein after boosting with multivalent MVA. Lines show GM with 95% confidence intervals. Dotted line represents seroconversion threshold. ****, p<0.0001 Friedman test.

5.7.3.49. Cellular Immune Response (Groups 1-3)

T cell responses to vaccination were measured by ex vivo interferon-gamma (IFNγ) ELISPOT at all time points and flow cytometry with intracellular cytokine staining (ICS) at the peak of the immune response after each vaccination. Prior to vaccination, ELISPOT responses in Groups 1-3 were below the level of detection of the assay in 72% of the participants (median, 50 spot-forming cells [SFCs] per million peripheral-blood mononuclear cells; 95% confidence interval, 53 to 63; Figure 75). Following priming vaccination with ChAd3-EBO Z, ELISpot responses peaked at day 14 (Figure 76, Figure 77), with median responses of 431 SFCs (interquartile range, 203 to 783) in group 1, 387 SFCs (interquartile range, 281 to 834) in group 2, and 693 SFCs (interquartile range [IQR], 348 to 866) in group 3. There was no significant difference in the magnitude of the immune response at any time point among the

different dose groups by Kruskal–Wallis test comparison. ELISPOT responses after boosting with MVA peaked after seven days after boosting vaccination at a median of 2068 SFC (IQR 1197-3447) and increased significantly more than three-fold compared with peak responses post prime (633 SFC, IQR 274-820, p<0.0001, 2-tailed Wilcoxon matched-pairs test, Figure 77). Responses were maintained 180 days after boosting at 498 SFC (IQR 207-905), significantly above non-boosted responses of 84 SFC (IQR 50-192, p<0.0001, Mann Whitney test). There was no relationship between the magnitudes of the antibody and T cell responses (Spearman's r=0.17, p=0.39).

T cell responses were further assessed in Groups 1-3 by intracellular cytokine staining at several post-vaccination time points. $CD4^+$ and $CD8^+$ T-cell responses to vaccination were assessed according to the secretion of interferon-γ, interleukin-2, or tumour necrosis factor α. At 28 days post-priming vaccination with ChAd3-EBO Z, cytokines were predominantly detected from CD4+ T cells, and cytokine expression tended to be higher in group 3 than in group 1 (p=0.06 by the Kruskal– Wallis test with Dunn's correction for multiple comparisons) (Figure 78). Positive GP-specific $CD4^+$ T-cell responses were detected in 50% of samples in group 1, 71% of samples in group 2, and 92% of samples in group 3. For $CD8^+$ T cells, the corresponding positivity rates were 20%, 64%, and 54%, respectively. Expression of the degranulation marker CD107a was also detected from $CD8^+$ T cells at day 28 after ChAd3 prime (Figure 79). After boosting with MVA, all volunteers had a positive $CD4^+$ and $CD8^+$ IFNγ T cell response and the median frequency of CD4+ T cells secreting IFNγ, IL2 or TNFα increased from 0.13% (IQR 0.004-0.19%) at day 28 to 0.20% (IQR 0.15-0.31%) at 7 days after MVA boosting (p<0.001, 2-tailed Kruskal-Wallis test, Figure 80). The increase in the frequency of cytokine-secreting $CD8^+$ T cells was even

more pronounced, from 0.004% (IQR 0.004-0.09%) at day 28 to 0.25% (IQR 0.10-0.65%) at M+7 ($p<0.001$, Kruskal-Wallis test). Expression of the degranulation marker CD107a on $CD8^+$ T cells increased five-fold after boosting ($p=0.003$, Kruskal-Wallis test, Figure 79). There was no significant effect of MVA dose on the magnitude of T cell responses measured by ELISPOT or ICS.

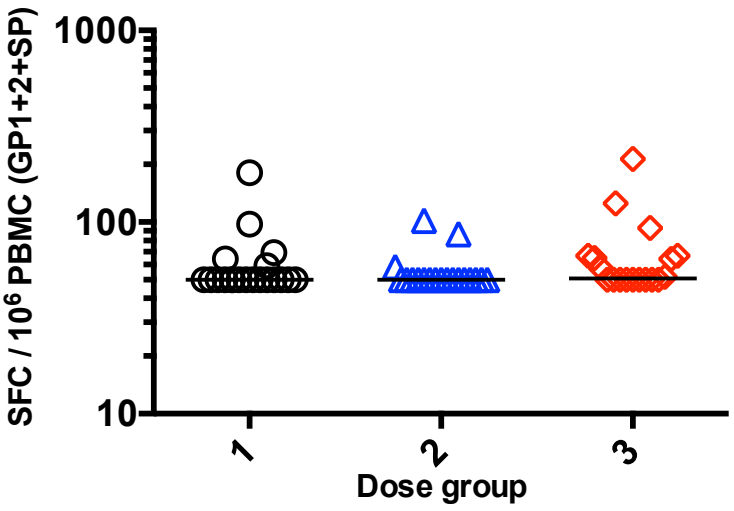


Figure 75: Group 1-3 ELISPOT responses at baseline prior to immunisation with ChAd3-EBO Z.

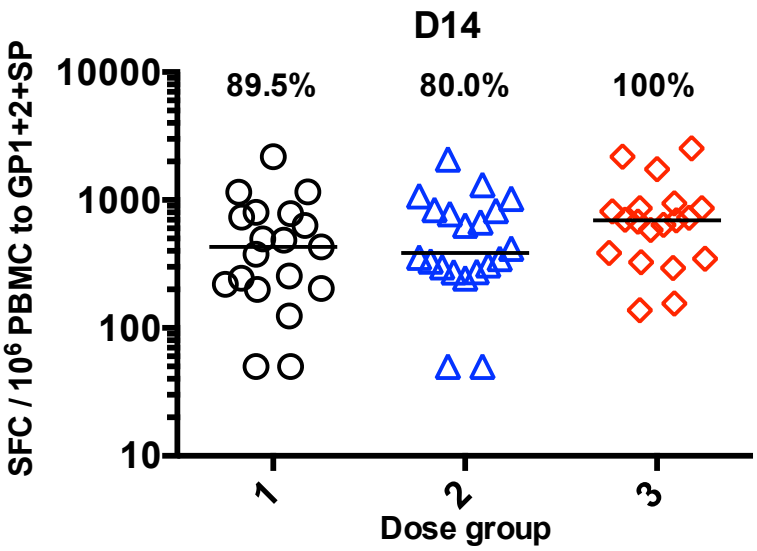


Figure 76: ELISPOT responses at the day 14 after vaccination with ChAd3-EBO Z. Percentages of participants who had a positive response (as indicated by a significant increase over the background pre-vaccination level) shown for each group.

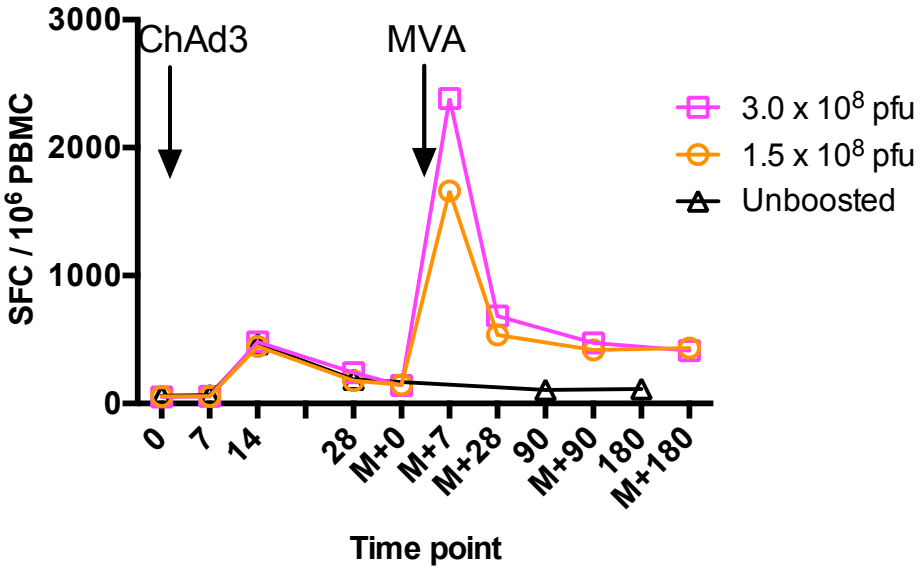


Figure 77: Group 1-3 Median T-cell responses to ChAd3 vaccination and MVA boosting as measured by ELISPOT assay at all time points.

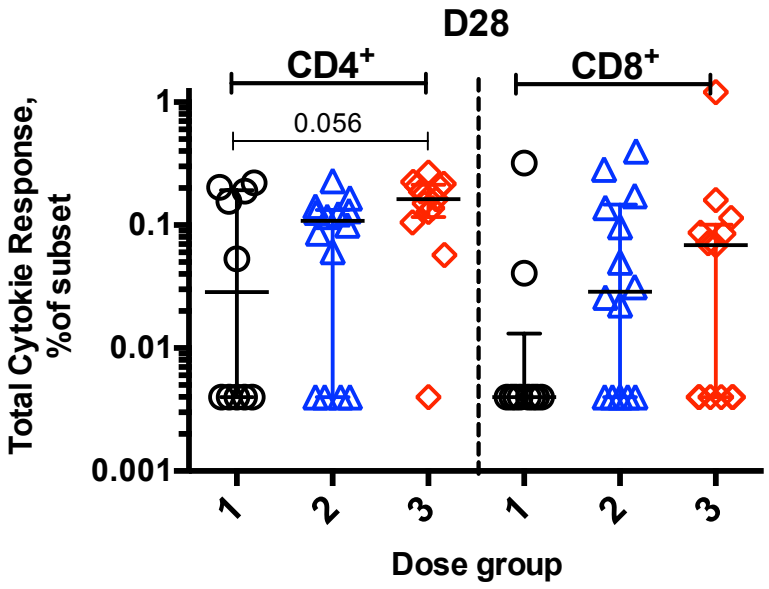


Figure 78: Intracellular cytokine staining (ICS) following ChAd3-EBO Z. Percentages of cells secreting interferon-γ, interleukin-2, and tumour necrosis factor α (TNF-α) from CD4+ and CD8+ T cells, as quantified for each dose group and shown as the percentage of cells expressing any one of the three cytokines.

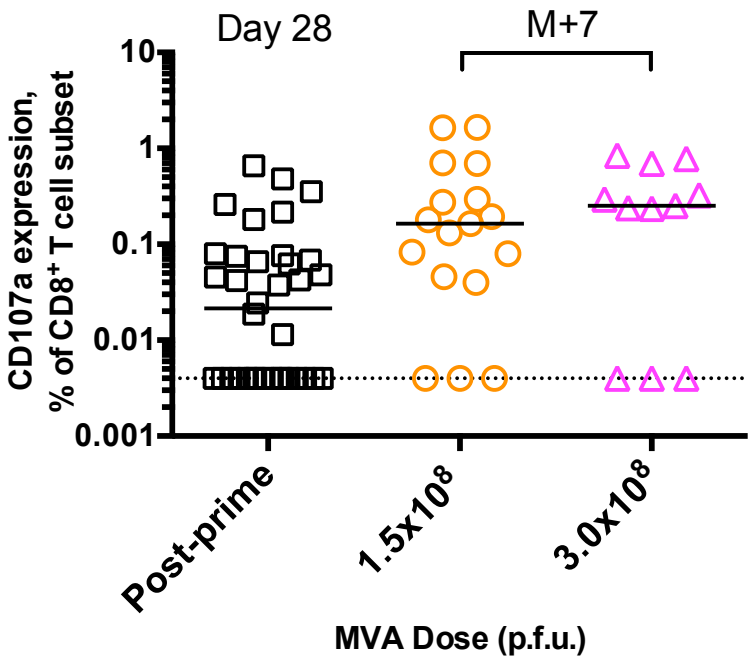


Figure 79: Expression of CD107a 28 days after priming or 7 days after boosting as measured by ICS. Horizontal lines indicate median values, and the dashed horizontal lines indicate the positive threshold.

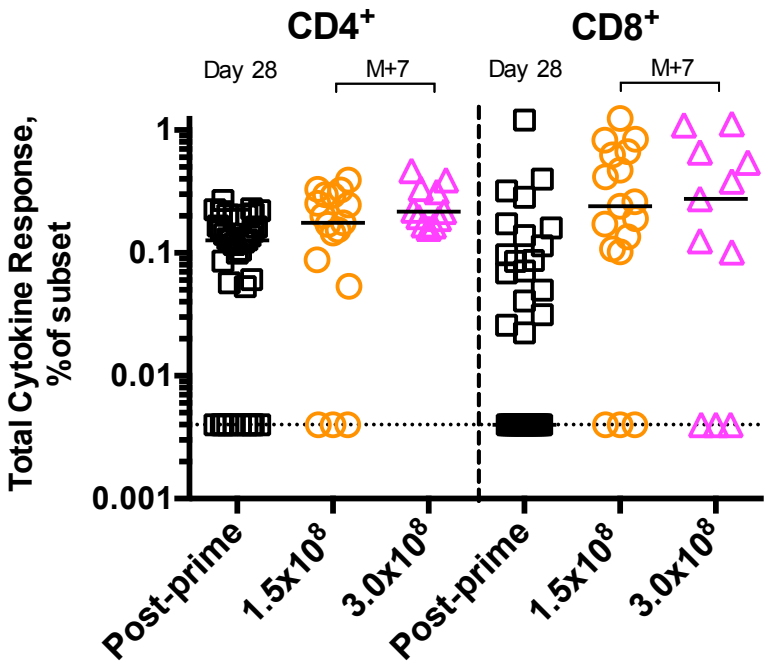


Figure 80: Total cytokine as measured by ICS at 28 days after priming with ChAd3 or 7 days after boosting with MVA according to dose. The secretion of IFN-γ, IL-2, and TNF-α by CD4+ and CD8+ T cells was quantified for each booster-dose group and ex- pressed as the frequency of cells expressing any one of the three cytokines.

5.7.3.50. Effect of variations in prime-boost interval on humoral immunogenicity

The interval between priming with ChAd3-EBO Z and boosting with MVA-BN® Filo had no effect on the magnitude of the antibody response (r = 0.20, p=0.30) (Figure 81). There was, however, a significant positive correlation between prime–boost interval and the neutralizing antibody titre, regardless of the MVA dose (r=0.72, p<0.001) (Figure 82). Conversely, a modest negative correlation was observed between prime-boost interval and peak ELISPOT response (r=-0.42, p=0.03, 2-tailed Pearson correlation, Figure 83).

The effect of reducing the prime-boost interval further to either one week (group 4, n=8) or two weeks (group 5, n=8) was assessed, using a ChAd3-EBO Z dose of 2.5 x

10^{10} vp and an MVA-BN® Filo dose of 1.5 x 10^{8}pfu in both groups. Analysis of antibody responses showed that reducing the prime-boost interval reduced the peak response post-MVA (Figure 84, $p<0.05$). Fourteen days after reduced interval boosting, antibody titres were significantly higher in each of group 4 and 5 compared with the unboosted group (Figure 85, $p<0.05$ for group 4, $p<0.01$ for group 5, Kruskal-Wallis test) with 88% of subjects in Groups 4 & 5, positive by ELISA compared with 55% in the group the unboosted group. Incorporating the data from groups 4 and 5 with boosted subjects from Groups 1-3, a significant positive correlation was observed between prime-boost interval and antibody response by standardized ELISA ($r=0.8$, $p<0.0001$, 2-tailed Spearman's test, (Figure 86). Neutralising antibody (nAb) titres to the live Ebolavirus were positive for all volunteers in group 4 and 50% of volunteers in group 5. The magnitude of the nAb titres was much lower than with longer intervals, however, confirmed by a strong positive correlation between prime-boost interval and nAb titre (Figure 87, $r=0.56$, $p=0.0007$, 2-tailed Spearman's correlation).

5.7.3.51. Effect of variations in prime-boost interval on cellular immunogenicity

In Groups 4 & 5, ELISPOT responses in both groups still peaked at seven days after boosting (Figure 88) and when analysing Groups 1-5, a slight negative correlation between prime-boost interval and peak T cell immunogenicity was observed ($r= -0.3$, $p=0.04$, 2-tailed Spearman's test). In addition the median ELISPOT response for groups boosted with either a 1 (Group 4), 2 (Group 5) or 3 to 10 week (Groups 1-3) interval for those primed with 2.5 x 10^{10} vp ChAd3 and boosted with 1.5 x 10^{8} pfu of

MVA-BN® Filo were compared. There were no significant differences between groups at either seven (Figure 90 Kruskal-Wallis test p=0.279) or 28 days (Figure 90 Kruskal-Wallis test p=0.3443) post MVA. Frequencies of cytokine-secreting $CD4^+$ T cells, as measured by ICS, were higher with two-week prime-boost interval than a 3-10 week interval ($p<0.01$, Kruskal-Wallis test, Figure 92). There was a slightly higher frequency of cytokine-secreting CD8+ T cells after a one-week prime boost interval ($p<0.05$, Kruskal-Wallis test).

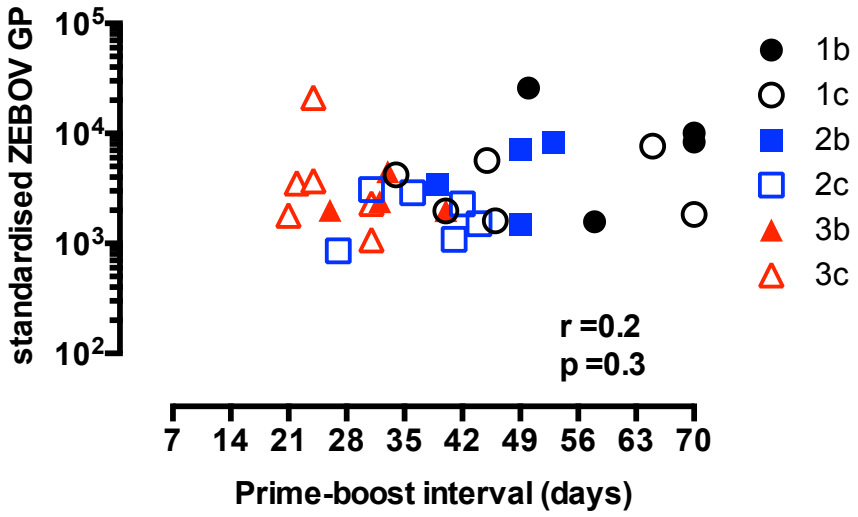


Figure 81: Effect of prime-boost interval on titres of IgG against ZEBOV glycoprotein as measured by standardised ELISA. Symbols: Circles Group 1 received $1x10^{10}$ vp ChAd3-EBO Z; Squares Group 2 $2.5x10^{10}$ vp; Triangles Group 3 $5x10^{10}$ vp; Closed symbols were boosted with $3x10^8$pfu MVA-BN® Filo; Open symbols received $1.5x10^8$pfu

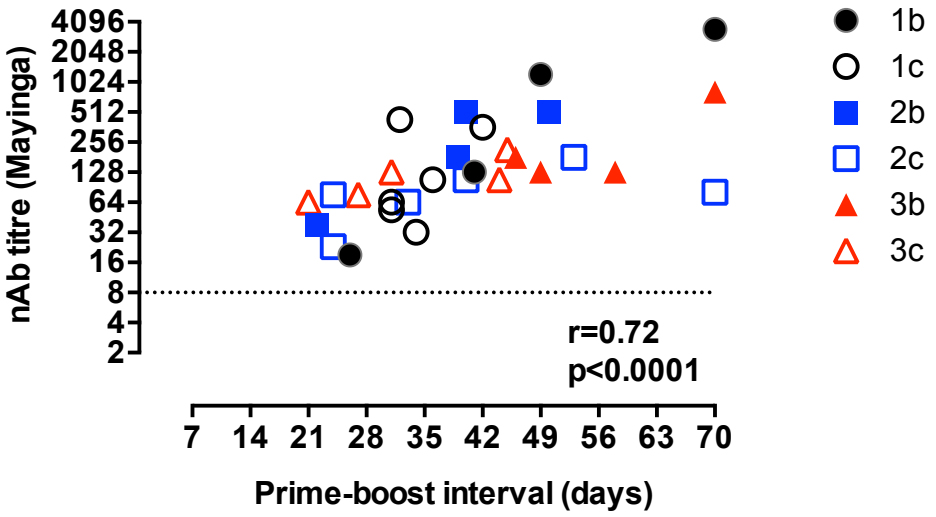


Figure 82: Association between neutralising antibody titres to live Ebolavirus virus and prime-boost interval. Symbols are per Figure 54. All correlations performed at fourteen days post-boost using 2-tailed Spearman test, n=29.

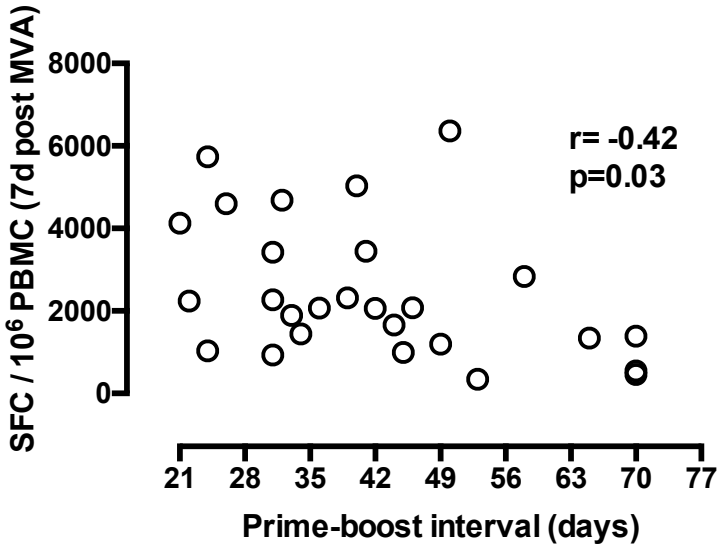


Figure 83: Relationship between the prime– boost interval and the peak ELISPOT response 7 days after MVA vaccination, as calculated by means of a two-tailed Spearman's test

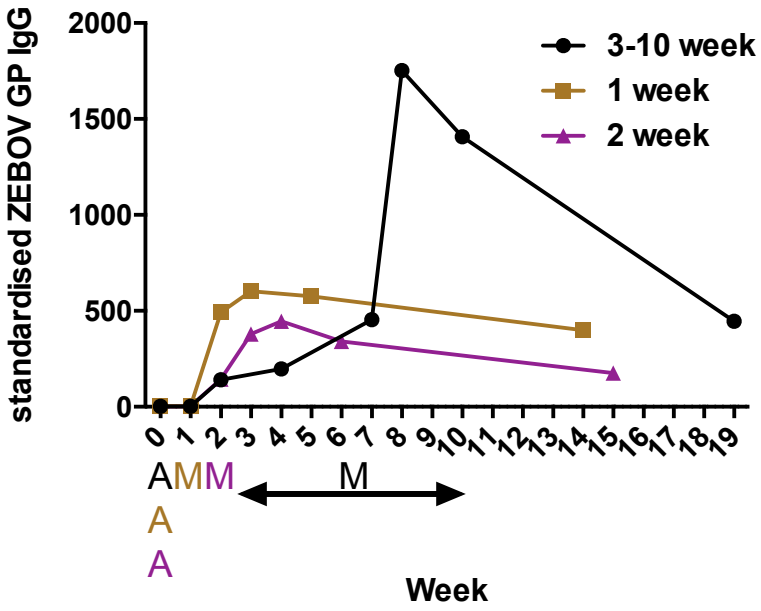


Figure 84: Geometric mean time course of anti-glycoprotein IgG levels measured with the ADI ELISA assay after vaccination with 2.5x1010vp ChAd3-EBO Z GP ("A") and boosting with 1.5x108 pfu MVA BN® Filo ("M"). The Y-axis units are optical density (OD) without subtraction of pre-vaccination titres.

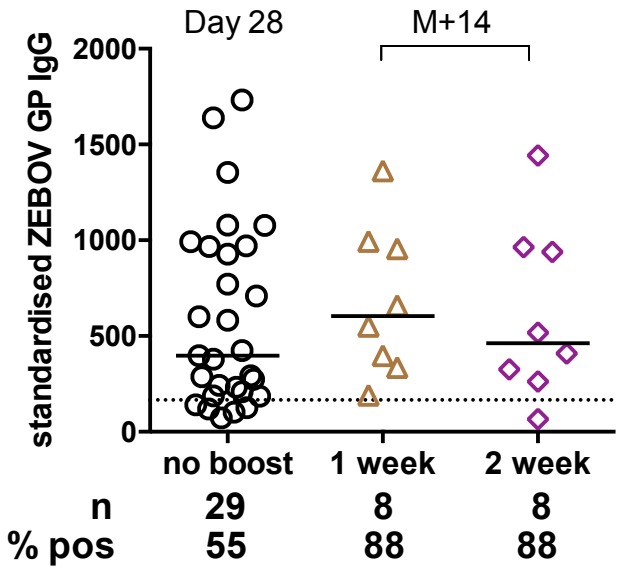


Figure 85: Individual responses one month after priming (Day 28), or 2 weeks after boosting (M+14) in volunteers receiving either no boost, or boosted with MVA after 1 week (Group 4) or 2 week (Group 5) interval.

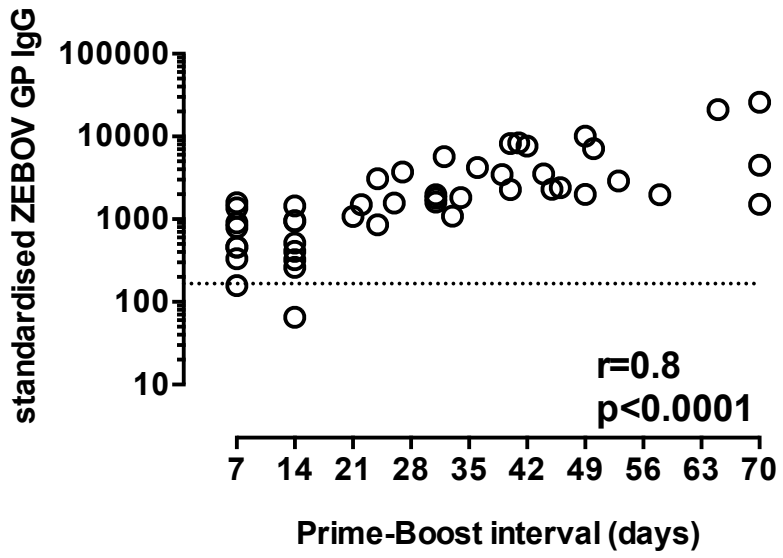


Figure 86: Effect of prime boost interval on anti- glycoprotein IgG titres for Groups 1-5 at fourteen days post boost (M+14) by 2-tailed Spearman test. The dotted line represents the positive threshold (OD+0.561), calculated from the mean + 3SD of the day 0 responses for all volunteers.

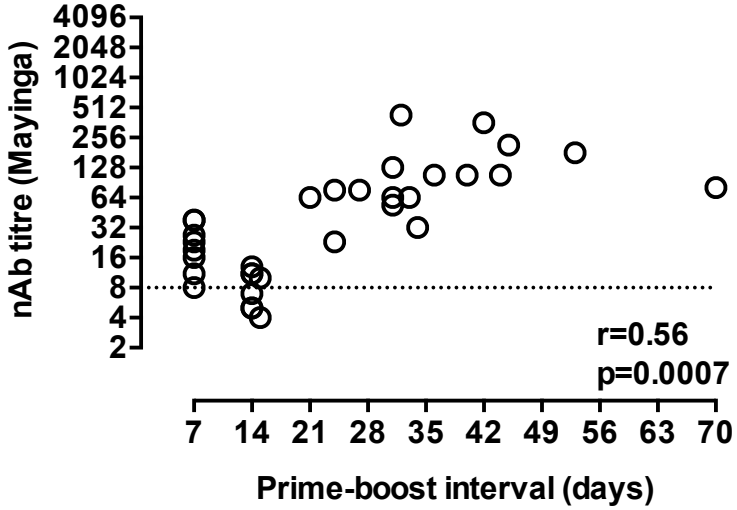


Figure 87: Effect of reduced prime boost interval on neutralising antibody titres at fourteen days post boost (M+14) by 2-tailed Spearman test.

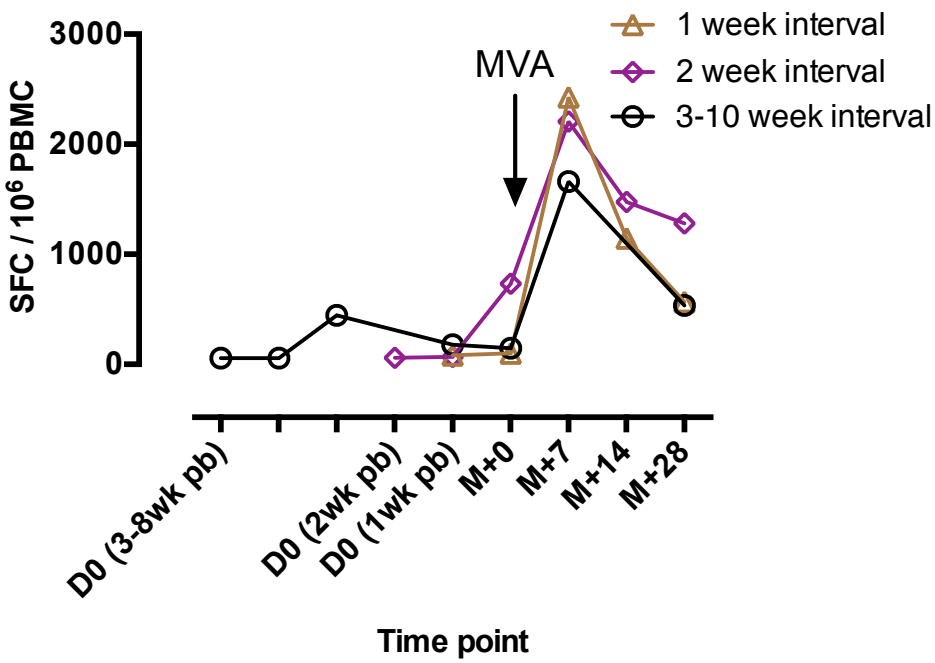


Figure 88: Effect of reduced prime–boost interval on cellular immunogenicity. The results are shown as median T-cell responses on ELISPOT assay, as measured in SFCs per million PBMCs.

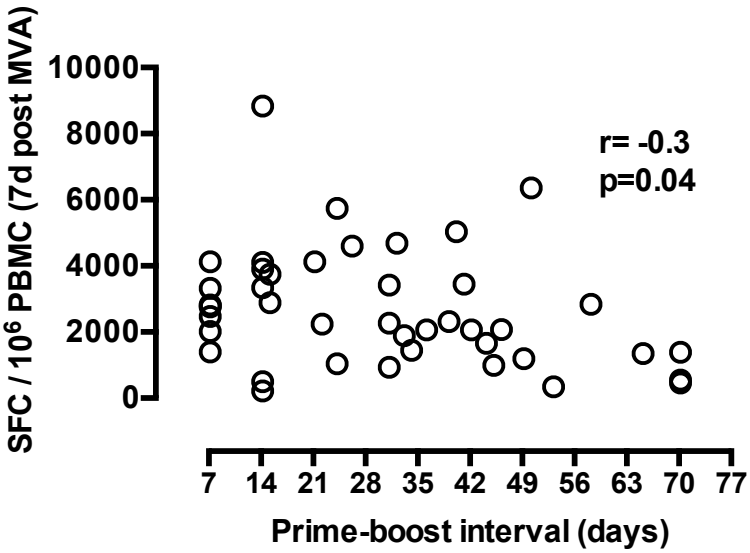


Figure 89: Correlation between length of prime–boost interval and peak cellular immunogenicity by ELISPOT at 7 days after boosting with MVA (M+7).

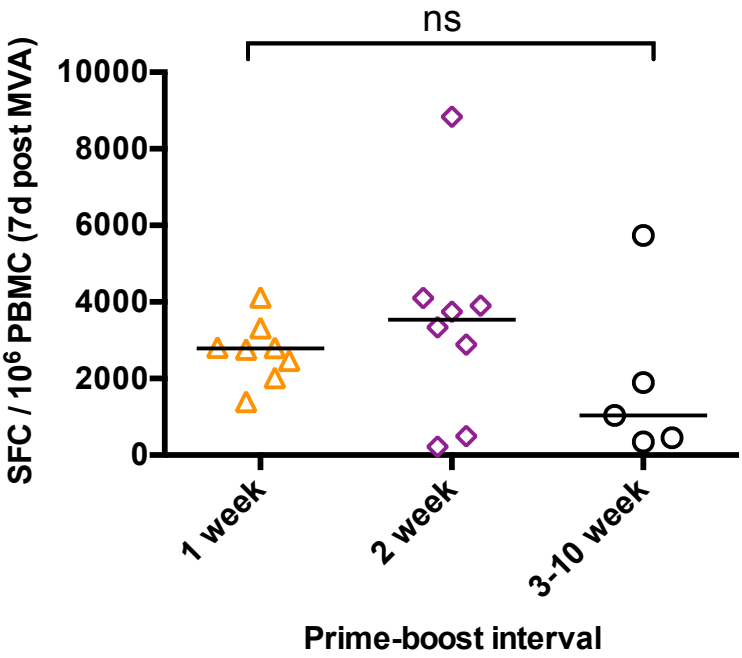


Figure 90: Individual ELISPOT responses to summed glycoprotein peptide pools at 7 days after MVA boost in subjects receiving 2.5x10^{10} vp ChAd3-EBO Z and 1.5x10^{8} pfu of MVA-BN® Filo with a 1 week (Group 4), 2 week (Group 5) and 3-10 week (Group 2c) prime-boost interval.

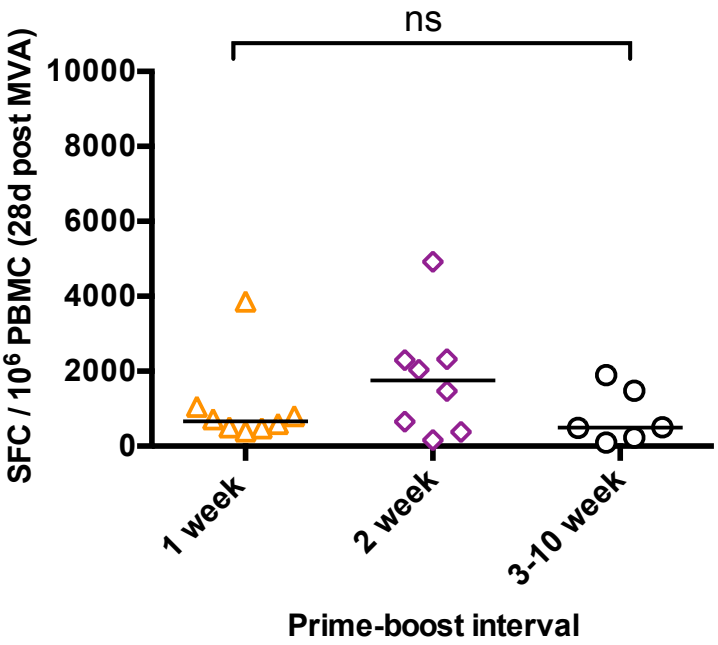


Figure 91: Individual ELISPOT responses to summed glycoprotein peptide pools at 28 days after MVA boost in subjects receiving 2.5x10^{10} vp ChAd3-EBO Z and 1.5x10^{8} pfu of MVA-BN® Filo with a 1 week (Group 4), 2 week (Group 5) and 3-10 week (Group 2c) prime-boost interval.

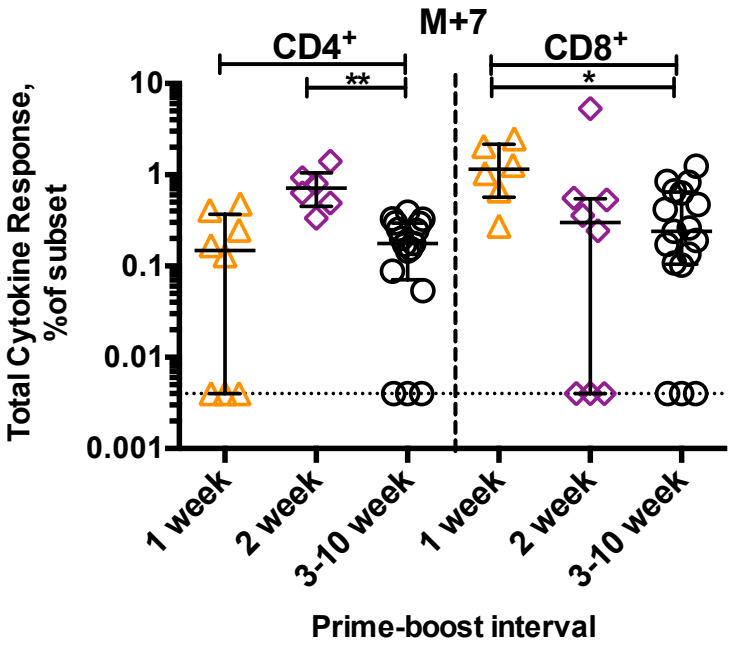


Figure 92: Flow cytometry with intracellular cytokine staining at seven days after boosting. IFNγ, IL2 and TNFα-secretion from CD4+ and CD8+ T cells was quantified for each prime-boost interval group and expressed as the proportion expressing any one of the three cytokines. Lines and error bars show median and inter-quartile ranges.

5.8. Discussion

This is the first human trial of the Ebolavirus vaccines ChAd3-EBO Z and MVA-BN® Filo either alone or as part of a heterologous prime-boost regimen. In this trial, the safety profile of both the ChAd3 and MVA vectors appears satisfactory. The great majority of local and systemic adverse events were mild, self-limiting and short-lived. This is similar to the safety profile of other MVA vaccines and other simian adenoviral vectors assessed clinically.[67,75,79,154,175-180] To date, and including this study, safety data has been published on over 300 subjects who have received the ChAd3-EBO Z vaccine, and approximately 180 who have received MVA-BN® Filo either after ChAd3-EBO Z or adenovirus serotype 26 (Ad26) prime without reports of any serious safety concerns.[181-183] The low rate of febrile reactions, in particular, is an

important asset for any potentially deployable filovirus vaccine. In the field, emergency infection control measures in Ebola treatment centres (ETCs) resulted in the cohorting of patients into Ebola holding units (EHUs) if they presented with fever ≥37.5°C and a positive Ebolavirus contact history.[184] Thus a low rate of febrile reactions is potentially important to reduce the risk of unnecessary admission to EHUs and subsequent nosocomial transmission of EVD.[185]

A notable aspect of this trial was the remarkable speed with which it was set up initiated and conducted, without compromising ethical, regulatory or scientific integrity. Input and prioritisation from several groups and agencies allowed for a successful application for funding, the filling and labelling to trial of the vaccines, submission and approval of applications to the regulatory and ethical bodies, completion of contractual arrangements, and initiation of this clinical trial in approximately one month (early August 2014 to early September 2014). This was matched by an exceptional level of public interest, enthusiasm and willingness to volunteer which made recruitment and vaccination swift and efficient. The input and prioritisation of this trial by various groups in response to declaration of an international public health emergency was key to rapid progress and highlighted that streamlining and adaptation of key processes is a likely necessity for clinical trials of drugs and vaccines in future potential outbreak scenarios. The prompt accrual of safety data from this trial allowed initiation of immunisation with the same vaccines in a trial in Bamako, Mali in early October.[181] In previous trials of some viral vectored vaccines, reduced immunogenicity has been observed in African populations compared to the responses in northern Europeans.[177,186] This highlights the

importance of immunogenicity assessments in West Africa for a vaccine intended for use in this population.

Immunogenicity assessments in this trial included measurements of Ebola Zaire glycoprotein-specific antibodies by ELISA, neutralizing antibody (nAb) assays against live Ebolavirus, IFN-γ ELISPOT and flow cytometry assays performed on fresh cells. These *ex-vivo* T cell assays provide a more sensitive measure of T cell immunogenicity not subject to diminution by PBMC cryopreservation. Dose of either ChAd3-EBO Z or MVA-BN® Filo had little effect on any measure of immunogenicity, with the exception of slightly higher nAb titres measured in the highest MVA dose group. Following ChAd3 prime antibody titres measured by ELISA peaked at day 28 after vaccination and were higher than would have been expected based on prior studies of antibody responses with other inserts in chimpanzee adenoviruses.[175,176] The boosting ability of MVA, particularly to enhance T cell responses, has been described pre-clinically and clinically for several diseases.[67,79,178,187] In this trial, substantial enhancements of antibody and T cell immunogenicity to levels comparable with ChAd3-immunised macaques protected from Ebola virus challenge five weeks post vaccination were observed [188]. In addition, MVA boosted responses were substantially higher than immune responses generated by single doses of either ChAd3, or Adenovirus 5 vaccines encoding the same antigen,[135,148] and boosting with MVA induced high levels of neutralising antibodies that correlated with the overall increased magnitude of antibody titres in IgG ELISA assays.

Only a single vaccine, the recombinant vesicular stomatitis virus expressing the Zaire Ebola glycoprotein (rVSV-ZEBOV) entered efficacy evaluation during the 2014 West-African Ebola outbreak. Recently published efficacy data reports 100% vaccine efficacy in >4500 Ebola virus contacts in the immediate vaccination group compared with the delayed vaccination group (95% CI 68·9–100·0, p=0·0045; endpoint of Ebolavirus disease occurring ≥10 days after randomisation)[189]. No immunogenicity data was generated during this trial, however comparison of neutralizing antibody (nAb) titres generated by rVSV ZEBOV in European Phase I trials show that a single dose of ChAd3-EBO Z generates similar nAb titres to a single dose of rVSV-ZEBOV, but boosting with MVA-BN® Filo increases 9-fold over prime alone.

Rapid induction of vaccine induced protective immunity is desirable for an Ebolavirus vaccine especially for use in outbreak settings. In this trial, substantial humoral and cellular immunogenicity was induced with prime boost intervals as short as one week. This is the shortest interval yet assessed in heterologous prime-boost trials. Such shorter interval regimes may facilitate vaccine deployment for the use of prime-boost vaccines.

Pre-clinical studies in macaques have shown that $CD8^+$ T cell depletion markedly reduces the efficacy of an adenovirus vaccine against lethal Ebolavirus challenge.[134] In the study presented here, a higher proportion of $CD4^+$ compared to $CD8^+$ T cells was observed in humans after ChAd3 prime, but this pattern was subsequently reversed to greater levels of $CD8^+$ T cell following MVA boosting.

A single dose of ChAd3 can induce 100% protection in macaques shortly after vaccination, and partial longer-term protection,[152] and a single dose of rVSV-ZEBOV

has been shown to induce high levels of protection acutely in humans.[189] Complete protection against lethal Ebolavirus challenge has been demonstrated in macaques at 10 months after vaccination when using a ChAd3 prime and MVA boost, but boosting with either the priming (ChAd3) vector or an alternative simian adenovirus serotype (ChAd63) vector was effective in inducing partial durable efficacy only.[152] The substantial boosting of immunological responses after MVA observed in the trial presented here, together with this pre-clinical data, strongly suggest that a two dose, heterologous prime boost regimen using ChAd3 and MVA may be favourable in achieving high level, durable efficacy.

There may be roles for both single dose and prime-boost regimens. High level, durable efficacy is particularly desirable for protecting populations against future epidemics, whereas single dose vaccines may prove preferable for the shorter-term efficacy required in outbreak settings, where issues of vaccine availability and feasibility of delivery may be paramount. It is important to note however, that this trial demonstrates that shorter (≥1 week) prime boost intervals are still effective at inducing strikingly potent CD8+ T cell immunogenicity just 2 weeks post priming. In addition, short prime boost intervals induce higher antibody responses than with single dose vaccination, albeit to lower levels than with longer prime-boost intervals.

The striking enhancement of both $CD8^+$ T cells and neutralising antibodies induced by ChAd3-EBO Z and MVA-BN® Filo heterologous prime-boost vaccination makes a strong case for further evaluation in Phase II and III studies.

Chapter 6

Conclusions and Closing Remarks

6. Conclusions and Closing Remarks

6.1. Viral vectored vaccines for tropical infections

In the preceding chapters, I have presented data from 3 clinical trials of viral vectored vaccines targeting the tropical infectious diseases malaria and Ebola. At the time of writing there remains no licensed vaccine for either disease, however, this may not remain true for much longer. The work presented in Chapters 3 and 4 represent a contribution to decades of prior scientific endeavour in the field of malaria vaccinology. The work presented in Chapter 5, however, is a contribution to a recent dramatic surge of interest in the field of vaccines that target emerging pathogens, including Ebola virus, that has occurred in response to the devastating 2014 Ebolavirus outbreak in West Africa. All studies presented in this thesis add further evidence to the argument that vaccines utilising a chimpanzee adenovirus and/or MVA vectors are safe, well tolerated and immunogenic. This work also contributes further compelling evidence that a heterologous prime-boost regimen using a chimpanzee adenovirus vectored prime, followed by an MVA vectored boost is an effective strategy for generating high level specific immunogenicity. It is unclear from the results of the VAC059 study (Chapter 4), however, whether anti-vector immunity to MVA may have implications for immunogenicity in a vaccine strategy employing multiple vaccine types with different antigenic targets and 2 consecutive doses of MVA. Viral vectors make attractive platforms for vaccines. In the case of emerging pathogens, similar vectors with existing manufacturing and safety profiles can be rapidly adapted to contain different antigenic inserts targeting novel pathogens, or strains of existing pathogens, and scale up of manufacture is rapid. In

the case of malaria, the high level of induced specific T cells is attractive, particularly to target the intracellular, and asymptomatic liver stage of the disease. There has previously been concern with viral vectors in liquid, or lyophilised formulation, that stability of the vaccines, especially at high temperature, may be an issue.[190,191] Increasingly, there is recognition of the need for thermostable vaccines for low and middle income countries.[191-196] Recent encouraging data has been presented, however, that reports stability of both the Ad26.ZEBOV and MVA-BN® Filo vaccines at 2-8°C for at least 12 months.[197] This has significant implications for these and other similar vectored vaccines, as it makes them compatible with typical cold chain requirements and suitable for deployment in remote parts of Africa.

6.2. Anti-vector immunity

Preceding the work in this thesis, considerable effort was made to develop viral vectored vaccines that could overcome the issue of anti-vector immunity, particularly for adenoviruses, as seroprevalence of certain adenovirus serotypes can be high.[198-211] In addition, the work presented in this thesis has postulated a possible deleterious effect of anti-MVA antibodies in the context of a vaccine regimen that contains 2 doses of MVA. This raises questions for the utility of a single vector (or a single, dual vector platform in the case of heterologous prime-boost) as a platform for multiple vaccines for different diseases. As an example, most people who would benefit from an Ebolavirus vaccine, would also benefit from a malaria vaccine. If a malaria vaccine using viral vectors has been introduced into the EPI, then it is possible that pre-existing anti-vector immunity may negatively impact on the effectiveness of a subsequently delivered Ebola vaccine if the same vectors are used.

A strategy for potentially circumventing this problem is to use different serotypes of adenovirus (eg. ChAd3 vs. ChAd63), but it is not yet clear what impact this may have on efficacy. Although pre-existing neutralising antibodies to certain human adenoviruses have been shown not to cross-neutralise chimpanzee adenoviruses,[212] it is not yet clear whether cross neutralisation between different chimpanzee adenoviral vectors may occur in humans. Immunogenicty induced by vaccination with replication-deficient vectors is likely to be weaker and of shorter duration than that raised by natural infection, however an alternative strategy to avoid the complications of anti-vector immunity would be to use adenoviruses, other than chimpanzee, that also have low prevalence in man. Bovine, canine, ovine, porcine and macaque adenoviruses have all been assessed as gene delivery platforms.[213] Another strategy to counter the effect of anti-vector immunity would be to stagger the immunisations, so that subsequent vaccination using the same vector as previously received, but encoding a different antigen was not given at the peak of anti-vector immunity. For example, separate the malaria and Ebola vaccines by 12 months or longer.

6.3. Implications for malaria vaccines

The results of the VAC055 (Chapter 3) study in which viral vectors expressing the liver stage antigen ME-TRAP were administered in a staggered regimen with the pre-liver stage recombinant subunit vaccine RTS,S/AS01B are encouraging for further evaluation of both multi-stage and multi-platform vaccine regimens for malaria. Data from the subsequent VAC059 trial (Chapter 4), however, unveil potentially critical issues with either same-site concomitant administration, or with a regimen that

contains 2 booster doses of MVA ME-TRAP, and this issue will need careful consideration when designing future studies. A focus of future work should be to determine an optimum dosing schedule and administration method for a vaccine regimen that incorporates a pre-liver stage subunit vaccine in adjuvant and viral vectors encoding lever stage antigens. The expensive, and resource intensive CHMI component may not be necessary in the first instance. Regimens with potential for high efficacy could be identified initially, based on immunogenicity.

Whilst CHMI studies are useful to identify possible candidates to take forward into field efficacy studies, they also have significant limitations. CHMI studies are both expensive and resource intensive, due in part to the rigorous safety oversight that must be employed. This, in turn, puts considerable restrictions on the number of volunteers that can undergo CHMI concurrently. All 3 CHMI exercises presented in this thesis generated sterile efficacy results that were statistically significant when compared with unvaccinated control subjects, but not on any comparison between vaccine groups. This is mainly a consequence of the limited numbers of target subjects in each vaccine group, but was also compounded by the higher than expected vaccine efficacy observed in each RTS,S/AS01B alone vaccine arm. Consideration should be given to future CHMI trial design regarding potential strategies to increase statistical power to detect differences in sterile efficacy between vaccine groups, assuming that practical limitations on the total number of concurrent subjects undergoing CHMI remain. Possible strategies in pursuit of this aim could include CHMI delivered not at the peak of vaccine induced immunogenicity (ie. several weeks or months after the final vaccine dose), or temporally dividing a larger overall study group, thereby conducting a series of

staggered vaccination cohorts and CHMI events under identical conditions with subsequent pooling of efficacy data.

In October 2015, the WHO convened the Strategic Advisory Group of Experts (SAGE) on Immunisation and the Malaria Policy Advisory Committee (MPAC) to review the future of RTS,S.[214] Following this, SAGE/MPAC jointly recommended large-scale pilot implementation of RTS,S to occur in children aged 5 to 17 months in 3 moderate-to-high transmission settings. The recommendation was subsequently adopted by the WHO in January 2016 and in April 2017, the trial sites were announced as Ghana, Malawi and Kenya, to begin vaccinating in 2018.[215]

6.4. Implications for Ebola vaccines

The field of Ebola vaccine development has seen unprecedented acceleration since autumn 2014, when the work in this thesis began. There is now a considerable body of phase 1 and phase II clinical trial data of Ebola vaccines that include ChAd3-EBO Z,[181,182,216] the bivalent ChAd3-EBO,[148] MVA-BN® Filo,[181,183,197,216] human adenovirus serotype 5,[217,218] and serotype 26 (Ad26.ZEBOV)[183,219] vectors both encoding the Zaire Ebola GP, and a recombinant vesicular stomatitis virus encoding the Zaire Ebola glycoprotein (rVSV-ZEBOV).[174,220-222] In addition, the rVSV-ZEBOV vaccine has been evaluated in a Phase III ring vaccination trial that took place in Guinea during the last few months of disease transmission in the 2014 West African Ebola outbreak and the final results have now been published with very encouraging efficacy data.[189] In this trial 2119 EVD contacts, and contacts of contacts, were immediately vaccinated and the outcomes compared with 2041 contacts and contacts of contacts who were vaccinated 21 days after randomisation. In the immediate vaccination cohort, no

cases of EVD occurred vs. 16 cases in the delayed vaccination group (100% VE [95% CI 68·9–100·0, p=0·0045]). In Chapter 5, there is a comparison of neutralising antibody titres generated by rVSV-ZEBOV, ChAd3-EBO Z or ChAd3-EBO Z boosted with MVA-BN® Filo, which shows comparable peak titres after a single dose of ChAd3-EBO Z, but a 9-fold increase after boosting with MVA-BN® Filo. Since the completion of this work, investigators evaluating rVSV-ZEBOV have also published specific T cell responses to their vaccine, with extremely low rates of $CD8^+$ T cell responses against the Ebola GP at peak time points in all dose groups as measured by both ELISPOT and intracellular cytokine staining. Given the high efficacy of rVSV-ZEBOV and apparent superior immunogenicity in many measures of ChAd3-EBO Z/MVA-BN® Filo, further evaluation in efficacy trials would be warranted in the event of future outbreaks. This may be difficult to justify ethically, given the high mortality of Ebolavirus disease, but important concerns about rVSV-ZEBOV remain unanswered, such as durability of vaccine induced protection and methods to mitigate poor vaccine stability at high temperatures[223] in areas of the world where maintenance of cold chain is challenging.

The work presented in this thesis is encouraging for viral vectored vaccines against Ebola and other outbreak viruses, including other filoviruses many of which are in the early stages of development currently.

7. APPENDIX

7.1. Schedule of procedures (ie. Trial visits blood tests etc.)

7.1.1. Schedule of Procedures for VAC055 (Chapter 3)

		S	RTS,S/AS01 (1)			ChAd63 ME-TRAP			RTS,S/AS01 (2)				RTS,S/AS01 (3)			MVA ME-TRAP			C-1
Attendance number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Timeline (days)			0	1	7	14	15	21	28	29	35	42	56	57	63	70	71	77	83
Window (days)		-90	-	*	±2	±2	*	±2	±2	*	±2	±2	±2	*	±2	±2	*	±2	-
Inclusion / Exclusion criteria		X	X			X%			X				X			X%			X
Informed Consent Questionnaire		X																	
Informed consent		X																	
Medical History		X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Physical Examination		X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Urinalysis		X																	
Electrocardiogram		X																	
β-HCG urine (women only)		X	X			X%			X				X			X%			X
Review contraindications		X	X			X%			X				X			X%			X
Vaccination			X			X%			X				X			X%			
Physical Observations^		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs reviewed			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card provided			X			X%			X				X			X%			
Diary card collected					X			X			X				X			X	
HLA typing (mL)			4																
HBV,HCV,HIV (mL)		5																	
Haematology (mL)		2			2			2			2		2		2	2		2	2
Biochemistry (mL)**		3			3			3			3		3		3	3		3	3
Anti- *P. Falciparum* serology (mL)$		2																	
Immunology			90			30			10		50	30	10			30		70	90
RNA Analysis			2.5	2.5		2.5	2.5		2.5	2.5			2.5	2.5		2.5	2.5		2.5
Blood Film / PCR																			2
Blood volume per visit (mL)		12	96.5	2.5	5	32.5	2.5	5	12.5	2.5	55	30	17.5	2.5	5	37.5	2.5	75	99.5
Cumulative blood volume (mL)	**Group 1**	**12**	**108.5**	**111**	**116**	**148.5**	**151**	**156**	**168.5**	**171**	**226**	**256**	**273.5**	**276**	**281**	**318.5**	**321**	**396**	**495.5**
	Group 2						**148.5**	**148.5**	**161**	**163.5**	**218.5**	**248.5**	**266**	**268.5**	**273.5**	**311**	**311**	**311**	**410.5**

Table 41: VAC055 Schedule of clinical reviews pre CHMI for Groups 1 & 2 (Windows refer to time since last visit); Grey columns are visits for Group 1 only; S = screening visit, (x) = If considered necessary, emphasising any acute complaints; * window = -18hrs to +48 hours. ^ Physical observations includes blood pressure, pulse and temperature, height and weight, however height and weight will only be measured

at C-1; Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Magnesium & Cholesterol, however Magnesium and cholesterol will only be measured at screening;$ Anti-*P.falciparum* serology may be tested at screening in certain individuals to help determine prior malaria exposure; % Group 1 only

	Group 3 & 4, and any sterilely protected Group 1 &2 Volunteers Undergoing Re-challenge			VAC045 volunteer undergoing re-challenge	
	S	C-1		S	C-1
Attendance number	1	2		1	2
Timeline (days)		0			0
Window (days)	-90	-2		-90	-2
Inclusion / Exclusion criteria	X	(X)		X	(X)
Informed consent	X			X	
Medical History	X	(x)		X	(x)
Physical Examination^	X	(x)		X	(x)
Urinalysis	X			X	
Electrocardiogram	X			X	
β-HCG urine (women only)	X	X		X	X
Review contraindications	X	X		X	X
Physical Observations^	X	X		X	X
HLA typing (mL)		4[%]			
HBV,HCV,HIV (mL)	5			5	
Haematology (mL)	2	2		2	2
Biochemistry* (mL)	3	3		3	3
Anti- *P. Falciparum* serology (mL)[$]	2[%]				
RNA analysis[@]		2.5			2.5
Immunology		90			90
Blood Film / PCR		2			2
Blood volume per visit (mL) – Group 3 & 4	12	101			
Blood volume per visit (mL) – Group 1 &2 Re-challenge	10	99.5			
Blood volume per visit (mL) – VAC045 volunteer undergoing re-challenge				10	99.5
Cumulative blood volume (mL) – Group 3 & 4	**12**	**113**			
Cumulative blood volume (mL) – any sterilely protected Group 1 & 2 volunteers undergoing re-challenge	**10**	**109.5**			
Cumulative blood volume (mL) – VAC045 volunteer undergoing re-challenge				**10**	**109.5**

Table 42: VAC055 Schedule of clinical reviews group 3 & 4 pre CHMI, Any Sterilely Protected Group 1&2Volunteers Undergoing repeat CHMI, & the VAC045 volunteer undergoing repeat CHMI. (Windows refer to time since last visit). S = screening visit, (x) = If considered necessary, emphasising any acute complaints, * Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Magnesium & Cholesterol, however Magnesium and cholesterol will only be measured at screening ^ Physical observations includes blood pressure, pulse and temperature, height and weight, however height and weight will only be measured at C-1.[$] Anti-*P.Falciparum* serology may be tested at screening in certain individuals to help determine prior malaria exposure.[@] Group 1 & 2 volunteers undergoing re-challenge only[%] Group 3 & 4 volunteers only

Timeline (days in relation to CHMI)	C	C6.5	C7[&]	C8-C14	C15-C23	C35	C90	C+150
Window (days)	0	0	0	0	0	±5	±14	±14
Medical History	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Clinical Assessment	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Urinary β H-CG[£]		(x)	(x)	(x)	(x)			
Physical Observations[%]	X	X	X	X	X	X	X	
Medic Alert Card Given to Volunteers	X							
Local & systemic events	X	X	X	X	X	X	X	X[€]
Treatment for Malaria		(x)	(x)	(x)	X			
Thick Smear / PCR for malaria		3	3 x 1, 2 x 1, 11 x 1	3 x 14	3 x 9			
Haematology (mL)				2^		2	2	
Biochemistry (mL)**				3^		3	3	
Immunology			40		50[$]	50	50	
Blood volume per visit(s) (mL)		3	56	47	77	55	55	
Cumulative blood volume (mL) – Group 1	495.5	498.5	554.5	601.5	678.5	733.5	788.5	788.5
Cumulative blood volume (mL) – Group 2	410.5	413.5	469.5	516.5	593.5	648.5	703.5	703.5
Cumulative blood volume (mL) – any sterilely protected Group 1 & 2 volunteers undergoing re-challenge	109.5	112.5	168.5	215.5	292.5	347.5	402.5	402.5
Cumulative blood volume (ml)- Group 3 & 4	113	116	172	219	296	351	406	406
Cumulutive blood volume – VAC045 volunteer undergoing re-challenge	109.5	112.5	168.5	215.5	292.5	347.5	402.5	402.5

Table 43: VAC055 Schedule of Clinic Attendances Post CHMI: All Volunteers (Windows refer to time since last visit). (x) = If considered necessary, emphasising any acute complaints. [£] Urinary β HCG will be performed in female volunteers prior to commencing anti-malaria treatment. [%] Physical observations includes blood pressure, pulse and temperature. [&] There will be three study visits on Day 7, beginning at 8-10am. There will be a 6-8 hour interval between visits. Blood will be taken for PCR at all three visits (10ml at the third visit), and for Blood film at the first and third visits. ** Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin & Liver Function Tests. ^Blood will be drawn for haematology & biochemistry on C9 and within 24 hours of diagnosis. If a volunteer is slide positive before C9 then blood will be drawn for biochemistry and haematology

within 24 hours of diagnosis and then not again until C35. $ Blood will be drawn for immunology on C+21 (50mls) if persistently slide-negative. @ Group 1&2 volunteers only € SAEs only will be collected on this visit

7.1.2. Schedule of Procedures for VAC059 (Chapter 4)

		S	Vaccination 1				Vaccination 2				Vaccination 3			C-1
Attendance number		1	2	3	4	5[%]	6	7	8	9	10	11	12	13
Timeline (days)			0	1	7	21	28	29	35	42	56	57	63	76
Window (days)		-90	-	*	±2	±2	±2	*	±2	±2	±2	*	±2	-
Inclusion / Exclusion criteria		X	X				X				X			X
Informed Consent Questionnaire		X												
Informed consent		X												
Medical History		X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Physical Examination		X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Urinalysis		X												
Electrocardiogram		X												
β-HCG urine (women only)		X	X				X				X			X
Review contraindications		X	X				X				X			X
Vaccination			X				X				X			
Physical Observations^		X	X	X	X	X	X	X	X	X	X	X	X	X
AEs reviewed			X	X	X	X	X	X	X	X	X	X	X	X
Diary card provided			X				X				X			
Diary card collected					X				X				X	
HLA typing (mL)			4											
HBV,HCV,HIV (mL)		5												
Haematology (mL)		2			2				2		2		2	2
Biochemistry (mL)**		3			3				3		3		3	3
Anti- *P. Falciparum* serology (mL)[$]		2												
Immunology			90			50[%]	10		50[%]	30	20		70[%]	90
RNA Analysis			2.5	2.5			2.5	2.5			2.5	2.5		2.5
Blood Film / PCR														2
Blood volume per visit (mL)		12	96.5	2.5	5	50[%]	12.5	2.5	5 (55[%])	30	27.5	2.5	5 (75[%])	99.5
Cumulative blood volume (mL)	**Group 1 & 2**	**12**	**108.5**	**111**	**116**	116	128.5	131	136	166	193.5	196	201	300.5
	Group 3 & 4					**166**	**178.5**	**181**	**236**	**266**	**293.5**	**296**	**371**	**470.5**

Table 44: VAC059 Schedule of clinical reviews pre CHMI for Groups 1-4 (Windows refer to time since last visit);Visits shaded grey are for group 3 & 4 volunteers only;

S = screening visit, (x) = If considered necessary, emphasising any acute complaints; * window = -18hrs to +48 hours. ^ Physical observations includes blood

pressure, pulse and temperature, height and weight, however height and weight will only be measured at C-1; Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Magnesium & Cholesterol, however Magnesium and cholesterol will only be measured at screening;[$] Anti-*P.falciparum* serology may be tested at screening in certain individuals to help determine prior malaria exposure; [%] Groups 3 & 4 only**

	Group 5 & 6, and any sterilely protected Group 1-4 Volunteers Undergoing Re-challenge	
	S	C-1
Attendance number	1	2
Timeline (days)		0
Window (days)	-90	-2
Inclusion / Exclusion criteria	X	(X)
Informed Consent Questionnaire	X[%]	
Informed consent	X	
Medical History	X	(x)
Physical Examination^	X	(x)
Urinalysis	X	
Electrocardiogram	X	
β-HCG urine (women only)	X	X
Review contraindications	X	X
Physical Observations^	X	X
HLA typing (mL)		4[%]
HBV,HCV,HIV (mL)	5	
Haematology (mL)	2	2
Biochemistry* (mL)	3	3
Anti- *P. Falciparum* serology (mL)[$]	2[%]	
RNA analysis		2.5[@]
Immunology		90
Blood Film / PCR		2
Blood volume per visit (mL) – Group 5 & 6	12	101
Blood volume per visit (mL) – Group 1-4 Re-challenge	10	99.5
Cumulative blood volume (mL) – Group 5 & 6	**12**	**113**
Cumulative blood volume (mL) – any sterilely protected Group 1-4 volunteers undergoing re-challenge	**10**	**109.5**

Table 45: VAC059 Schedule of clinical reviews for Group 5 & 6 pre CHMI and, any Sterilely Protected Group 1-4 Volunteers Undergoing repeat CHMI (Windows refer to time since last visit). S = screening visit, (x) = If considered necessary, emphasising any acute complaints, * Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Magnesium & Cholesterol, however Magnesium and cholesterol will only be measured at screening ^ Physical observations includes blood pressure, pulse and temperature, height and weight, however height and weight will only be measured at C-1. [$] Anti-*P.Falciparum* serology may be tested at screening in certain individuals to help determine prior malaria exposure. [@] Group 1-4 volunteers undergoing re-challenge only [%] Group 5 & 6 volunteers only

Timeline (days in relation to CHMI)	C	C+6.5	C+7[&]	C+8-C+14	C+15-C+25	C+35	C+90	C+170[@]
Window (days)	0	0	0	0	0	±7	±14	±14
Medical History	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Clinical Assessment	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Urinary β H-CG[£]		(x)	(x)	(x)	(x)			
Physical Observations[%]	X	X	X	X	X	X	X	
Medic Alert Card Given to Volunteers	X							
Local & systemic events	X	X	X	X	X	X	X	X[€]
Treatment for Malaria		(x)	(x)	(x)	X			
Thick Smear / PCR for malaria		3	3 x 1, 2 x 1, 11 x 1[&]	3 x 14	3 x 11			
Haematology (mL)				2^		2	2	
Biochemistry (mL)**				3^		3	3	
Immunology			40		50[$]	50	50	
Blood volume per visit(s) (mL)		3	56	47	83	55	55	
Cumulative blood volume (mL) – Group 1 & 2	300.5	303.5	359.5	406.5	489.5	544.5	599.5	599.5
Cumulative blood volume (mL) – Group 3 & 4	470.5	473.5	529.5	576.5	659.5	714.5	769.5	769.5
Cumulative blood volume (mL) – any sterilely protected Group 1-4 volunteers undergoing re-challenge	109.5	112.5	168.5	215.5	298.5	353.5	408.5	408.5
Cumulative blood volume (ml)- Group 5 & 6	113	116	172	219	302	357	412	412

Table 46: VAC059 Schedule of Clinic Attendances Post CHMI: All Volunteers (Windows refer to time since last visit). (x) = If considered necessary, emphasising any acute complaints. [£] Urinary β HCG will be performed in female volunteers prior to commencing anti-malaria treatment. [%] Physical observations includes blood pressure, pulse and temperature. [&] There will be three study visits on Day 7, beginning at 8-10am. There will be a 6-8 hour interval between visits. Blood will be taken for PCR at all three visits (10ml at the third visit), and for Blood film at the first and third visits. ** Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin & Liver Function Tests. ^Blood will be drawn for haematology & biochemistry on C+9 and within 24 hours of diagnosis. If a volunteer is slide positive before C+9 then blood will be drawn for biochemistry and haematology within 24 hours of diagnosis and then not again until C+35. [$] Blood will be drawn for immunology on C+23 (50mls) if persistently slide-negative. [@] Group 1-4 volunteers only and only following initial challenge [€] SAEs only will be collected on this visit

7.1.3. Schedule of Procedures for EBL01 (Chapter 5)

Attendance	1*	2	3	4	5[&]	6	7	8	9
Timeline (days)	≤90	0	1	7	10	14	28	90	180
Window period (days)			±1	±1	±2	±2	±7	±7	±14
Inclusion/Exclusion criteria	X								
Informed Consent		X							
Physical exam	X	(X)	(X)	(X)	(X)[&]	(X)	(X)	(X)	(X)
Inspection of vaccination site		X	X	X					
Physical observations^	X	X	X	X	X	X	X	(X)	(X)
Medical history	X	(X)	(X)	(X)	(X) [&]	(X)	(X)	(X)	(X)
Urinalysis	X								
βHCG – Urine test[%]	X	X							
Review contraindications	X	X							
Study Vaccination		X							
AEs & SAEs reviewed[@]		X	X	X	X[&]	X	X	X	X
Diary Card provided		X							
Diary card collection				X					
Oral fluid collection		X				X	X		
Telephone contact									
HLA typing		4							
HIV, HBV, HCV	5								
Haematology	2	2	2	2		2	2	2	2
Biochemistry[£]	3	3	3	3		3	3	3	3
Coagulation screen + fibrinogen	3	3	3	3		3	3		
Exploratory Immunology[$]		90		70	30[&]	50	90	90	90
Intracellular RNA		3	3	3					
Daily Volume (mL)	**13**	**105**	**11**	**81**	**30**	**58**	**98**	**95**	**95**
Max. Cumulative Volume Groups 1 and 2 (mL)	**13**	**118**	**129**	**210**	**210**	**268**	**366**	**461**	**556**
Max. Cumulative Volume Group 3 (mL)	**13**	**118**	**129**	**210**	**240**	**298**	**396**	**491**	**586**

Table 47: EBL01 Schedule of attendances for all subjects unless enrolled into subgroups 1b, 1c, 2b, 2c, 3b or 3c; * = Screening visit; (X) = if considered necessary; ^ = Physical observations include pulse, blood pressure, temperature; [%] = females only; [£] = Biochemistry will include Sodium, Potassium, urea, Creatinine, Albumin, ALT, and Bilirubin;@ = solicited AEs will be collected until day 7 following vaccination, unsolicited AEs and SAEs will be collected until the end of the study. See sections 2.6.2-2.6.4. for further details [$] = Exploratory Immunology will include ELISA, IFN-γ T cell ELISPOT, B cell assays, and further experimental assays to be confirmed at a later date. These will be outlined in detail in the separate immunomonitoring plan; [&] = Group 3 only Visits and window periods are relative to the most recently given vaccination

Attendance	1	2	3	4	5[&]	6	7	8	9	10	11	12	13	14
Visit	S	C0	C1	C7	C10	C14	C28	M0	M1	M7	M14	M28	M90	M180
Timeline (days)	≤90	0	1	7	10	14	28	42	43	49	56	70	132	222
Window period (days)			±1	±1	±2	±2	±7	+28/-21	±1	±1	±2	±7	±7	±14
Inclusion/Exclusion criteria	X													
Informed Consent		X												
Physical exam	X	(X)	(X)	(X)	(X)[&]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Inspection of vaccination site		X	X	X										
Physical observations^	X	X	X	X	X	X	X	X	X	X	X	X	(X)	(X)
Medical history	X	(X)	(X)	(X)	(X)[&]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urinalysis	X													
βHCG – Urine test[%]	X	X						X						
Review contraindications	X	X						X						
Study Vaccination		X						X						
AEs & SAEs reviewed[@]		X	X	X	X[&]	X	X	X	X	X	X	X	X	X
Diary Card provided		X						X						
Diary card collection				X						X				
Oral fluid collection		X				X	X	(x)[?]			(x)[?]	(x)[?]		
Telephone contact														
HLA typing		4												
HIV, HBV, HCV	5													
Haematology	2	2	2	2		2	2	2	2	2	2	2	2	2
Biochemistry[£]	3	3	3	3		3	3	3	3	3	3	3	3	3
Coagulation screen + fibrinogen	3	3	3	3		3	3	3	3	3	3	3	3	3
Exploratory Immunology[$]		90		70	30[&]	50	90	50		90	30	50	90	60
Intracellular RNA		3	3	3				3	3	3				
Daily Volume (mL)	**13**	**105**	**11**	**81**	**30**	**58**	**98**	**61**	**11**	**101**	**38**	**58**	**98**	**68**
Max. Cumulative Volume Groups 1 and 2 (mL)	**13**	**118**	**129**	**210**	**210**	**268**	**366**	**427**	**438**	**539**	**577**	**635**	**733**	**801**
Max. Cumulative Volume Group 3 (mL)	**13**	**118**	**129**	**210**	**240**	**298**	**396**	**457**	**468**	**569**	**607**	**665**	**763**	**831**

Table 48: EBL01 Schedule of attendances for all subjects enrolled into subgroups 1b, 1c, 2b, 2c, 3b or 3c; S = Screening visit; (X) = if considered necessary; ^ = Physical observations include pulse, blood pressure, temperature; [%] = females only; [£] = Biochemistry will include Sodium, Potassium, urea, Creatinine, Albumin, ALT, and Bilirubin;@ = solicited AEs will be collected until day 7 following vaccination, unsolicited AEs and SAEs will be collected until the end of the study. See sections 2.6.2-2.6.4. for further details [$] = Exploratory Immunology will include ELISA, IFN-γ T cell ELISPOT, B cell assays, and further experimental assays to be confirmed at a later date. These will be outlined in detail in the separate immunomonitoring plan; [&] = Group 3

only; Visits and window periods are relative to the most recently given vaccination; [?] = only for subjects who did not have oral fluid collected at C0, C14, C28.

Attendance	1	2	3	4	5	6	7	8	9
Visit	S	C0	C1	M0	M1	M7	M14	M21	M90
Timeline (days)	≤90	0	1	7	8	14	21	28	97
Window period (days)			±1	±1	±2	±2	±2	±7	±14
Inclusion/Exclusion criteria	X								
Informed Consent		X							
Physical exam	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Inspection of vaccination site		X	X	X					
Physical observations^	X	X	X	X	X	X	X	X	X
Medical history	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urinalysis	X								
βHCG – Urine test[%]	X	X		X					
Review contraindications	X	X		X					
Study Vaccination		X		X					
AEs & SAEs reviewed[@]		X	X	X	X	X	X	X	X
Diary Card provided		X		X					
Diary card collection				X		X			
Oral fluid collection				(X)	(X)	X	X	(X)	(X)
HLA typing		4							
HIV, HBV, HCV	5								
Haematology	2	2		2		2		2	
Biochemistry[£]	3	3		3		3		3	
Coagulation screen + fibrinogen	3	3		3		3			
Exploratory Immunology[$]		90		70		90	50	50	90
Intracellular RNA		3	3	3	3	3			
Daily Volume (mL)	**13**	**105**	**3**	**81**	**3**	**101**	**50**	**55**	**90**
Max. Cumulative Volume	**13**	**118**	**121**	**202**	**205**	**306**	**356**	**411**	**501**

Table 49: EBL01 Schedule of attendances for subjects enrolled into Groups 4 & 6; S = Screening visit; (X) = if considered necessary; ^ = Physical observations include pulse, blood pressure, temperature; [%] = females only; [£] = Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, ALT, and Bilirubin; @ = solicited AEs will be collected until day 7 following vaccination, unsolicited AEs and SAEs will be collected until the end of the study. See sections 2.6.2-2.6.4. for further details [$] = Exploratory Immunology will include ELISA, IFN-γ T cell ELISPOT, B cell assays, and further experimental assays to be confirmed at a later date. These will be outlined in detail in the separate immunomonitoring plan; Visits and window periods are relative to the most recently given vaccination.

Attendance	1	2	3	4	5	6	7	8	9	10
Visit	S	C0	C1	C7	M0	M1	M7	M14	M28	M90
Timeline (days)	≤90	0	1	7	14	15	21	28	42	104
Window period (days)			±1	±1	±2	±1	±2	±2	±7	±14
Inclusion/Exclusion criteria	X									
Informed Consent		X								
Physical exam	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Inspection of vaccination site		X	X	X						
Physical observations^	X	X	X	X	X	X	X	X	X	X
Medical history	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Urinalysis	X									
βHCG – Urine test[%]	X	X			X					
Review contraindications	X	X			X					
Study Vaccination		X			X					
AEs & SAEs reviewed[@]		X	X	X	X	X	X	X	X	X
Diary Card provided		X			X					
Diary card collection				X			X			
Oral fluid collection					(X)	(X)	X	X	(X)	(X)
HLA typing		4								
HIV, HBV, HCV	5									
Haematology	2	2		2			2	2	2	
Biochemistry[£]	3	3		3			3	3	3	
Coagulation screen + fibrinogen	3	3		3			3			
Exploratory Immunology[$]		90		70	50		90	50	50	90
Intracellular RNA		3	3	3	3	3	3			
Daily Volume (mL)	**13**	**105**	**3**	**81**	**53**	**3**	**101**	**55**	**55**	**90**
Max. Cumulative Volume	**13**	**118**	**121**	**202**	**255**	**258**	**359**	**414**	**469**	**559**

Table 50: Schedule of attendances for subjects enrolled into Groups 5 & 7; S = Screening visit; (X) = if considered necessary; ^ = Physical observations include pulse, blood pressure, temperature; [%] = females only; [£] = Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, ALT, and Bilirubin;@ = solicited AEs will be collected until day 7 following vaccination, unsolicited AEs and SAEs will be collected until the end of the study. See sections 2.6.2-2.6.4. for further details [$] = Exploratory Immunology will include ELISA, IFN-γ T cell ELISPOT, B cell assays, and further experimental assays to be confirmed at a later date. These will be outlined in detail in the separate immunomonitoring plan; Visits and window periods are relative to the most recently given vaccination.

7.2. Immunology & PCR methods

Colleagues who performed each assay are detailed in the statements of work preceding each chapter, and they have generously provided the methods as detailed below. Some or all of these methods have been published in the supplementary appendices in the relevant study publications [160,216,224] & *Rampling et al.* Submitted npj Vaccines 2017.

7.2.1. Malaria Immunogenicity Assays (VAC055 and VAC059)

7.2.1.52. *Ex-vivo* Interferon-γ (IFN-γ) Enzyme-Linked Immunosorbent Spot (ELISPOT) assays

Ex vivo (18 hour stimulation) ELISPOT assays were performed using Multiscreen IP ELISPOT plates (Millipore), human IFNγ SA-ALP antibody kits (Mabtech) and BCIP NBT-plus chromogenic substrate (Moss Inc). Cells were cultured in RPMI (Sigma) containing 1% sterile filtered Penicillin-Streptomycin (Sigma), 1% L-Glutamine (Sigma) and 10% heat-inactivated, sterile-filtered foetal calf serum, previously screened for low reactivity (Labtech International). Antigens were tested in triplicate with 250,000 PBMC added to each well of the ELISPOT plate. Plates were counted using an AID automated ELISPOT counter (AID Diagnostika GmbH, algorithm C), using identical settings for all plates and counts were adjusted only to remove artefacts. Responses to the negative control were always < 85 SFC per million PBMC. Responses were considered positive if four times greater than the negative control for the corresponding sample.

7.2.1.53. Peptides for T cell Assays

TRAP peptides were 20 amino acids in length, overlapping by 10 amino acids (Neopeptide), assayed in 6 pools of 7-10 peptides at 10 µg/mL. CSP peptides were 15 amino acids in length, overlapping by 11 amino acids (a kind gift of the Malaria Department, US Naval Medical Research Center), assayed in 3 pools of 3-12 peptides at 10 µg/mL. Responses were averaged across duplicates (VAC055) or triplocates (VAC059), responses in unstimulated (negative control) wells were subtracted and then responses in individual pools were summed for each strain of the TRAP antigen or for CSP. ME responses were assayed in a single pool and peptide pool configurations are shown in **Table 52**, **Table 53** and Table 54. Staphylococcal enterotoxin B (0.02 µg/mL) and phytohaemmagglutinin-L (10µg/mL) were used as a positive control.

7.2.1.54. Flow cytometry with Intracellular Cytokine Staining (ICS)

Samples for flow cytometry were stimulated in parallel with the *ex-vivo* ELISPOT using fresh PBMC. After overnight stimulation, samples were stained and acquired the same day on the Jenner Institute LSR II flow cytometer. Responses to CSP and TRAP were assessed using a single pool of peptides for each antigen. Peptide sequences are described in Table 52, Table 53 and **Table 54**. Details of staining and analysis are given in Table 55. A hierarchical gating strategy was applied for analysis.

Responses were assessed by a 9-colour staining panel on freshly isolated PBMC, in parallel with ELISPOT assays. Aliquots of 2×10^6 PBMC in 1 ml of medium containing anti-CD28 and anti-CD49d at 1 µg ml−1 (eBioscience) and CD107a-PeCy5 (1:500,

eBioscience) were stimulated with no antigen, a pool of 56 peptides spanning the T9/96 strain of the TRAP antigen (20mers overlapping by 10 amino acids, at 2 μg ml−1), a pool of 56 peptides spanning the 3D7 strain of the TRAP antigen (20mers overlapping by 10 amino acids, at 2 μg ml−1), a pool of 31 peptides spanning the CS antigen (15mers overlapping by 11 amino acids, at 2 μg ml−1) or a positive control, Staphylococcal enterotoxin B (Sigma, 1 μg ml−1) in 5 ml polystyrene FACS tubes for 18 hours at 37°C and 5% CO2. Brefeldin A and Monensin, both at 1 μg ml−1, were added for the last 16 h. Cells were incubated with a dead cell discrimination dye (AQUA 1:200, Invitrogen) for 20 minutes at room temperature. PBMC were permeabilised, then stained intracellularly at room temperature for 30 minutes with CD4-APC (1:25, eBioscience) CD14- and CD19-Pacific Blue (both 1:50, eBioscience), CD3-Alexa Fluor 700 (1:50, eBioscience), CD8-APC-Alexa Fluor 780 (1:10, eBioscience) and IFN-γ-FITC (1:100, eBioscience), IL-2-PE (1:50., eBioscience) and TNFα-Pe-Cy7 (1:500, eBioscience), then washed and fixed in 1% paraformaldehyde. Further details of monoclonal antibodies are given in Table 55. Compensation was performed using single-stained One-Comp beads (eBioscience) for monoclonal antibodies and ARC beads for AQUA (Life Technologies).

Acquisition was performed on the day of staining on a BD LSRII. Medians live CD3+ cell counts are shown in **Table 51**.

	Median live CD3+ cells acquired per sample	IQR
VAC055	501,000	(25%=361750, 75% = 627000
VAC059	675,000	IQR 506,250-765,000

Table 51: Median live CD3+ cells acquired per sample by trial with interquartile ratios (IQR

Data was prepared and analysis performed using FlowJo v9.6.2 (Treestar Inc). Cells were gated on lymphocytes, singlets, live $CD3^+$, CD8-$CD4^+$ or CD4-$CD8^+$ and then IFNγ, IL-2, TNFa and CD107a. Dead cells (AQUA+), monocytes ($CD14^+$) and B cells ($CD19^+$) were excluded from the analysis. All SEB stimulated PBMC gave a cytokine response >1%. Responses to peptide were determined after subtraction of the response in the unstimulated control for each sample, and considered positive if the count was >20 and frequency higher than the autologous unstimulated control and the lower limit of detection.

Peptide Name	**Peptide sequence T9/96**	**Peptide sequence 3D7**	**T9/96 Peptide Pool**	**3D7 Peptide Pool**
TRAP-1	MNHLGNVKYLVIVFLIFFDL		TT1-10	TD1-10
TRAP-2	VIVFLIFFDLFLVNGRDVQN		TT1-10	TD1-10
TRAP-3	FLVNGRDVQNNIVDEIKYSE	FLVNGRDVQNNIVDEIKYRE	TT1-10	TD1-10
TRAP-4	NIVDEIKYSEEVCNDQVDLY	NIVDEIKYREEVCNDEVDLY	TT1-10	TD1-10
TRAP-5	EVCNDQVDLYLLMCSGSIR	EVCNDEVDLYLLMCSGSIR	TT1-10	TD1-10
TRAP-6	LLMCSGSIRRHNWVNHAVP		TT1-10	TD1-10
TRAP-7	RHNWVNHAVPLAMKLIQQLN		TT1-10	TD1-10
TRAP-8	LAMKLIQQLNLNDNAIHLYV	LAMKLIQQLNLNDNAIHLYA	TT1-10	TD1-10
TRAP-9	LNDNAIHLYVNVFSNNAKEI	LNDNAIHLYASVFSNNAREI	TT1-10	TD1-10
TRAP-10	LNDNAIHLYVNVFSNNAKEI	SVFSNNAREIIRLHSDASKN	TT1-10	TD1-10
TRAP-11	IRLHSDASKNKEKALIIIRS	IRLHSDASKNKEKALIIIKS	TT11-20	TD11-20
TRAP-12	KEKALIIIRSLLSTNLPYGR	KEKALIIIKSLLSTNLPYGK	TT11-20	TD11-20
TRAP-13	LLSTNLPYGRTNLTDALLQV	LLSTNLPYGKTNLTDALLQV	TT11-20	TD11-20
TRAP-14	TNLTDALLQVRKHLNDRINR		TT11-20	TD11-20
TRAP-15	RKHLNDRINRENANQLVVIL		TT11-20	TD11-20
TRAP-16	ENANQLVVILTDGIPDSIQD		TT11-20	TD11-20
TRAP-17	TDGIPDSIQDSLKESRKLSD		TT11-20	TD11-20
TRAP-18	SLKESRKLSDRGVKIAVFGI		TT11-20	TD11-20
TRAP-19	RGVKIAVFGIGQGINVAFNR		TT11-20	TD11-20
TRAP-20	GQGINVAFNRFLVGCHPSDG		TT11-20	TD11-20
TRAP-21	FLVGCHPSDGKCNLYADSAW		TT21-30	TD21-30
TRAP-22	KCNLYADSAWENVKNVIGPF		TT21-30	TD21-30
TRAP-23	ENVKNVIGPFMKAVCVEVEK		TT21-30	TD21-30
TRAP-24	MKAVCVEVEKTASCGVWDEW		TT21-30	TD21-30
TRAP-25	TASCGVWDEWSPCSVTCGKG		TT21-30	TD21-30
TRAP-26	SPCSVTCGKGTRSRKREILH		TT21-30	TD21-30

TRAP-27	TRSRKREILHEGCTSEIQEQ	TRSRKREILHEGCTSELQEQ	TT21-30	TD21-30
TRAP-28	EGCTSEIQEQCEEERCPPKW	EGCTSELQEQCEEERCLPKR	TT21-30	TD21-30
TRAP-29	CEEERCPPKWEPLDVPDEPE	CEEERCLPKREPLDVPDEPE	TT21-30	TD21-30
TRAP-30	EPLDVPDEPEDDQPRPRGDN		TT21-30	TD21-30
TRAP-31	DDQPRPRGDNSSVQKPEENI	DDQPRPRGDNFAVEKPNENI	TT31-40	TD31-40
TRAP-32	SSVQKPEENIIDNNPQEPSP	FAVEKPNENIIDNNPQEPSP	TT31-40	TD31-40
TRAP-33	IDNNPQEPSPNPEEGKDENP	IDNNPQEPSPNPEEGKGENP	TT31-40	TD31-40
TRAP-34	NPEEGKDENPNGFDLDENPE	NPEEGKGENPNGFDLDENPE	TT31-40	TD31-40
TRAP-35	NGFDLDENPENPPNPDIPEQ	NGFDLDENPENPPNPPNPPN	TT31-40	TD31-40
TRAP-36	NPPNPDIPEQKPNIPEDSEK	NPPNPPNPPNPPNPPNPPNP	TT31-40	TD31-40
TRAP-37	*NONE*	PPNPPNPPNPDIPEQKPNIP	TT31-40	TD31-40
TRAP-38	DIPEQKPNIPEDSEKEVPSD	DIPEQKPNIPEDSEKEVPSD	TT31-40	TD31-40
TRAP-39	EDSEKEVPSDVPKNPEDDRE		TT31-40	TD31-40
TRAP-40	VPKNPEDDREENFDIPKKPE		TT31-40	TD31-40
TRAP-41	ENFDIPKKPENKHDNQNNLP		TT41-50	TD41-50
TRAP-42	NKHDNQNNLPNDKSDRNIPY	NKHDNQNNLPNDKSDRYIPY	TT41-50	TD41-50
TRAP-43	NDKSDRNIPYSPLPPKVLDN	NDKSDRYIPYSPLAPKVLDN	TT41-50	TD41-50
TRAP-44	SPLPPKVLDNERKQSDPQSQ	SPLAPKVLDNERKQSDPQSQ	TT41-50	TD41-50
TRAP-45	ERKQSDPQSQDNNGNRHVPN		TT41-50	TD41-50
TRAP-46	DNNGNRHVPNSEDRETRPHG		TT41-50	TD41-50
TRAP-47	SEDRETRPHGRNNENRSYNR		TT41-50	TD41-50
TRAP-48	RNNENRSYNRKYNDTPKHPE		TT41-50	TD41-50
TRAP-49	KYNDTPKHPEREEHEKPDNN		TT41-50	TD41-50
TRAP-50	REEHEKPDNNKKKGESDNKY		TT41-50	TD41-50
TRAP-51	KKKGESDNKYKIAGGIAGGL		TT51-57	TT51-57
TRAP-52	KIAGGIAGGLALLACAGLAY		TT51-57	TT51-57
TRAP-53	ALLACAGLAYKFVVPGAATP		TT51-57	TT51-57
TRAP-54	KFVVPGAATPYAGEPAPFDE		TT51-57	TT51-57
TRAP-55	YAGEPAPFDETLGEEDKDLD		TT51-57	TT51-57
TRAP-56	TLGEEDKDLDEPEQFRLPEE		TT51-57	TT51-57
TRAP-57	EPEQFRLPEENEWN		TT51-57	TT51-57

Table 52: TRAP peptide pool format Peptide sequences and residue numbers based on those of the *P. falciparum* clone T9/96 (GenBank no. CAA31440.1).

Peptide Name	Peptide sequence	Antigen
st8	MINAYLDKL	STARP
ls50	ISKYEDEI	LSA1
pb9	SYIPSAEKI	*P. berghei CSP*
ls8	KPNDKSLY	LSA1
cp26	KPKDELDY	CSP
ls6	KPIVQYDNF	LSA1
tr42/43	ASKNKEKALII	TRAP
tr39	GIAGGLALL	TRAP

cp6	MNPNDPNRNV	CSP
tr26	HLGNVKYLV	TRAP
ls53	KSLYDEHI	LSA1
tr29	LLMCSGSI	TRAP
csp	DPNANPNVDPNANPNV	CSP
38H BCG	QVHFQPLPPAVVKL	BCG
FTTp	QFIKANSKFIGITE	TT
cp39	YLNKIQNSL	CSP
la72	MEKLKELEK	LSA3
ex23	ATSVLAGL	EXP1
nanp	NANPNANPNANPNANP	CSP
trapAM	DEWSPCSVTCGKGTRSRKRE	TRAP

Table 53: ME peptide pool format

Peptide number	Amino acid sequence
1	MMAP DPNANPNANPN
2	NANP NANPNANPNAN
3	DPNA NPNANPNKNNQ
4	NPNA NPNKNNQGNGQ
5	NPNK NNQGNGQGHNM
6	NNQG NGQGHNMPNDP
7	NGQG HNMPNDPNRNV
8	HNMP NDPNRNVDENA
9	NDPN RNVDENANANS
10	RNVD ENANANSAVKN
11	ENAN ANSAVKNNNNE
12	ANSA VKNNNNEEPSD
13	VKNN NNEEPSDKHIK
14	NNEE PSDKHIKEYLN
15	PSDK HIKEYLNKIQN
16	HIKE YLNKIQNSLST
17	YLNK IQNSLSTEWSP
18	IQNS LSTEWSPCSVT
19	LSTE WSPCSVTCGNG
20	WSPC SVTCGNGIQVR
21	SVTC GNGIQVRIKPG
22	GNGI QVRIKPGSANK
23	QVRI KPGSANKPKDE
24	KPGS ANKPKDELDYA
25	ANKP KDELDYANDIE
26	KDEL DYANDIEKKIC
27	DYAN DIEKKICKMEK
28	DIEK KICKMEKCSSV
29	KICK MEKCSSVFNVV
30	MEKC SSVFNVVNSSI
31	KCSS VFNVVNSSIGL

Table 54: CSP Peptide sequences. Peptide sequences were based on *P. falciparum* clone 3D7 (GenBank no. X15363). Series of 15 amino acid sequences overlapping by 11 amino acids.

Antibody	Fluorochrome /Dye	Clone	Supplier	Product code	Final Dilution
	LIVE/DEAD (Amine reactive dye)	AQUA	Life Technologies	L34955	1:400
CD3	Alexa Fluor 700	OKT3	eBioscience	56-0037-42	1:100
CD4	Allophycocyanin (APC)	RPA-T4	eBioscience	17-0049-73	1:50
CD8	APC-eFluor780	RPA-T8	eBioscience	47-0088-42	1:25
CD14	eFluor 450	61D3	eBioscience	48-0149	1:100
CD19	eFluor 450	HIB19	eBioscience	48-0199	1:100
CD107a	PE-Cy5	eBioH4A3	eBioscience	15-1079-42	1:100
IFNγ	FITC	4S.B3	eBioscience	11-7319-82	1:200
IL-2	PE	MQ1-17H12	eBioscience	12-7029-82	1:100
TNFα	PE-Cy7	MAb11	eBioscience	25-7349-82	1:1000

Table 55: Antibodies used for flow cytometry. CD107a was added at the start of the 18-hour stimulation, LIVE/DEAD was surface-stained. All other markers were stained after permeabilisation.

7.2.1.55. IgG ELISAs

Antibody responses to CS and TRAP were measured by ELISA on serum samples at the Jenner Institute, UK. Antibody responses to CS were measured by IgG ELISA performed at the WRAIR International Reference Centre for Malaria Serology.

7.2.1.56. Total IgG Enzyme Linked Immunosorbent Assay (ELISA) to TRAP

Recombinant TRAP antigen was produced by transient transfection of HEK293E cells, using a method similar to that previously reported. [225,226] A transgene comprising the human tissue plasminogen activator secretory signal peptide fused in frame with the 3D7-clone TRAP ectodomain (lacking the native signal peptide, transmembrane domain and a run of PNP repeats stretching from P356 to P370) was codon-

optimised for mammalian expression (Life Technologies). The transgene cassette was cloned using the InFusion enzyme system (ClonTech) into the pENTR LPTOS plasmid backbone, [227] in which expression of the transgene is driven by an intron-containing CMV immediate early promoter, with additional in-frame C-terminal biotin acceptor peptide and Strep(II) tags. [228] Four days after polyethyleneimine mediated transfection of HEK293E cells, supernatant was harvested and affinity purified on a Streptactin affinity column (IBA Lifesciences). The resulting protein was >90% pure, as demonstrated by Coomassie Blue stained SDS-PAGE (data not shown).

Nunc-Immuno 96 well plates were coated with 0.5µg/mL of TRAP antigen in carbonate-bicarbonate coating buffer and left overnight at 4°C. Plates were washed 6x with PBS-Tween (PBS/T), then blocked with 1% BSA in PBS/T for 1 hour at room temperature (RT). Serum was diluted in PBS/T containing 0.2% BSA at concentrations of 1:100, 1:300, or 1:900, and added in triplicate. Serum samples from days A+0 A+*n* where n=number of days following ChAd63 ME-TRAP), A+28, A+56, A+63, C-1, C+7 and C+90 were analysed. Plates were incubated at RT for 2 hours then washed as before. A secondary antibody (goat anti-human whole IgG conjugated to alkaline phosphatase, Sigma) was added at a dilution of 1:1000 in PBS/T 0.2% BSA for 1 hour at RT. After a final wash, plates were developed by adding 4-nitrophenyl phosphate in diethanolamine buffer (Pierce).

A positive reference standard (made from pooled TRAP-positive serum) was used on each plate to give a standard curve. It was added in duplicate at an initial dilution of 1:100 (in PBS/T 0.2% BSA) and diluted 2-fold 10 times, starting with an arbitrary

value of 20 antibody units. Four blank wells (zero antibody units) were also designated. The optical density (OD) values were then fitted to a 4 parameter standard curve using SOFTmax PRO software. [229] An internal control was included on every plate in triplicate made up from a 1:400 dilution (in PBS/T 0.2% BSA) of the positive standard. OD was read at 405nm using an ELx800 microplate reader. Test sera antibody units were calculated from their OD values using the parameters estimated from the standard curve.

7.2.1.57. Total IgG Enzyme Linked Immunosorbent Assay (ELISA) to CS [230]

ELISA 96-well plates were coated with a synthetic peptide (Eurogentec) based on the repeat region of the PfCSP with the amino acid sequence CS(NANP)6C. The peptide was coated at a concentration of 0.2 µg/mL in a volume of 100 µL per well. Plates were placed inside a humidity chamber and incubated overnight (16 - 20 h) at 22°C. Plates were washed four times with 1xPBS (pH 7.4) containing 0.5% Tween-20 and blocked with 0.5% casein blocking buffer (Sigma) for 1 h at 22°C. Plates were washed four times and serially diluted samples were added and incubated at 22°C for 2 h. After washing four times, peroxidase labeled goat anti-human IgG (KPL) was added at a dilution of 1:4,000 and incubated at 22°C for 1 h. After washing four times, ABTS Peroxidase substrate (KPL) was added for development and incubated for 1 h at 22°C. The data were collected using Softmax Pro GXP, data were fit to a 4-parameter logistic curve, and the serum dilution at which the optical density was 1.0 (OD 1.0) calculated. To serve as a positive control, serum obtained from a volunteer participating in a Phase 1/2a challenge trial of R32NS181 formulated with alum was

used. [231] The individual had anti-PfCSP antibodies but was not protected (*personal communication from WRAIR serology laboratory*). Samples were considered positive if the difference between the post-immunisation OD 1.0 and the pre-immunisation OD 1.0 (net OD 1.0) was > 50 and the ratio of post- immunisation OD 1.0 to pre-immunisation OD 1.0 (ratio) was > 2. For example, if the OD 1.0 was 150 post-immunisation and 50 pre-immunisation, the net OD 1.0 would be 100, and the ratio of OD 1.0 post-immunisation to OD 1.0 pre-immunisation would be 3. This would be considered positive.

In the VAC059 study (Chapter 4), Kinetics of the NANP-specific response are shown for all volunteers receiving RTS,S/AS01B alone (groups 1 and 2 combined) and volunteers receiving RTS,S/AS01B with viral vectors (groups 3 and 4 combined) until the third vaccination where groups 2 and 4 received a fractional dose of RTS,S/AS01B and are shown separately (Figure 41). In further analyses, groups 1 and 2 (RTS,S/AS01B only), and groups 3 and 4 (RTS,S/AS01B with viral vectors) were combined as there were no significant differences between these groups on these assays.

7.2.1.58. CSP-specific IgG Avidity ELISA

IgG antibody avidity was assessed by sodium thiocyanate (NaSCN)-displacement ELISA. The assays were conducted as for total IgG ELISAs except that sera were individually diluted in casein to a level calculated to reach an OD405 of 1.0 (using total IgG EUs), and plated at 50uL/well in 16 wells of a 96 well plate. Plates were incubated for 2 hours at RT before chaotropic agent NaSCN was added in duplicate at increasing concentrations down the plate (0 to 7 Molar (M)). Plates were

incubated for 15 mins at RT before washing, incubated with secondary antibody and developed with the same conditions as the total IgG assay. The concentration of NaSCN required to reduce the OD405 to 50% of that in wells where no NaSCN (=IC50) was added was used as a measure of avidity.

7.2.1.59. Anti-MVA IgG ELISA

Anti-MVA ELISAs were conducted using serum taken at baseline (D0), D42 and C-1 for all volunteers. Pre-coated and pre-blocked 96-well ELISA plates were kindly donated by Dr. Huw Davies, University of California, Irvine. Plates were coated with MVA protein WR113/D8L, blocked with Casein/TBS, dried and stored at 4°C until use. Samples were diluted 1:200 in blocking buffer (1% Casein in TBS supplemented with 10% E. coli lysate). After incubating in blocking buffer at room temperature (RT) for 30 minutes to block anti-E. coli reactivity, samples were added to the plate in duplicate, 50uL/well. Plates were incubated for 45 minutes at RT before washing 6 times with PBS. Secondary antibody (goat anti-human IgG conjugated to Horseradish Peroxidase, ADI, H-HuG.211) was added 100uL/well at a dilution of 1:100 in PBS and plates were incubated at RT for 45 minutes. Plates were washed 6 times in PBS before adding 100uL of TMB substrate (ADI, 80091) per well and covering plates to protect from light. After 10 minutes, the reaction was stopped by adding 100uL/well of stop solution (ADI, 80101). Optical density (OD) was read at 450nm on an ELx800 microplate reader (Biotek) with Gen5 software (version 2.07).

7.2.1.60. Parasite Quantitative PCR (qPCR)

Blood for qPCR was collected at baseline and at timepoints following CHMI for qPCR in 2.0ml tubes containing EDTA before being filtered to reduce white cell content.

DNA was extracted from 0.5mL filtered blood using Qiagen Blood Mini Kit. 5µL of each extraction was used per assay (total eluate volume = 50µL) and was run in triplicate for qPCR (equivalent to 150µL blood directly assessed). Parasites per mL (p/mL) equivalent mean values were generated by a standard Taqman absolute quantitation, against a defined plasmid standard curve. This was conducted on an ABI StepOne Plus machine and v2.3 software using default Universal qPCR and QC settings, apart from the use of 45 cycles and 25µL reaction volume.

7.2.2. Ebola Immunogenicity Assays (EBL01)

7.2.2.61. ELISA assays

Anti-glycoprotein IgG responses to the Zaire and Sudan species of Ebolavirus were assessed using commercially available kits (AE 320620-1 and AE321600-1) from Alpha Diagnostics International, Texas, USA (ADI), according to the manufacturer's instructions with serum diluted at 1:500. Monoclonal antibodies were provided in the kit as a control to standardize between plates. Optical density (OD) was read at 450nm on an EL800 microplate reader (Biotek). Values are reported with and without the pre-vaccination OD subtracted and were converted to micrograms per ml using the reference serum provided by the manufacturer. Concentrations in ug/mL were calculated based on an average OD from 8 experiments of the positive control provided in the kit added undiluted to the plate (as per manufacturer's instructions) and based on the ug/mL value provided by the manufacturer. This concentration is 166ng/mL, which allowed calculation that 29.8ng of polyclonal Ebola GP-specific IgG in 100uL sample added to the well gives an OD of 1.0. The estimated ug/mL for each sample was then back-calculated using that conversion

factor. This calculation is an estimate limited the availability of only one dilution for the comparison rather than a fitted standard curve. The NIH ELISA was performed as described elsewhere[16]. One volunteer in group 3 (019) was excluded from the analysis of antibody responses due to high IgG responses to the glycoprotein prior to immunisation that could not be explained, despite repeating the assay.

7.2.2.62. ZEBOV antigen ELISA

EBOV and mock antigens were prepared from cell culture supernatants of ZEBOV-infected (6 d post infection) or uninfected (6 d post seeding) VeroE6 cells. Concentration of particles from the supernatant was performed via ultracentrifugation through a 20% sucrose cushion at 4°C and 25,000 rpm for 2 hours. Resulting pellets were resuspended in PBS containing 1% SDS. To inactivate EBOV, samples were boiled for 10 minutes before they were removed from BSL-4 facility of the Philipps University of Marburg. High binding single-break strip microtitre plates (Greiner bio-one, Cat.No. 705073 & 705075) were coated with 50 µl ZEBOV (Zaire Ebola Virus, Guinea isolate, C7, AccNo: KJ660347) or Mock antigen (both diluted 1:1000 in PBS, final protein concentration of viral antigen about 2 µg/ml) and incubated for 16-17 hours at 4°C. Further incubations were performed at room temperature. ELISA plates were washed three times with PBS/0.1% Tween®20 (PBST, Sigma Aldrich, P7949) and then blocked for 45 minutes with PBS containing 5% milk powder. Washing procedure was repeated three times with PBST. Sera of volunteers were diluted 1:200 in PBST containing 1% milk powder and allowed to react with EBOV and mock antigen for 1 hour. After washing plates three times with PBST, polyclonal rabbit anti-human IgG/HRP (DAKO, P0214) was used for detection.

Plates were washed two times with PBST and two times with PBS. Bound IgG was detected by SureBlue™ TMB Microwell Peroxidase substrate (KPL, 52-00-00). TMB Stop Solution (KPL, 50-85-04) was added after 10 minutes and optical density was determined at 450-620nm. A panel of positive and negative controls and standards was run each time the assay was performed to validate the experiment. Corrected OD values were calculated by subtracting mock OD values from values obtained by incubating the same serum with ZEBOV antigen.

The NIH ELISA to the Mayinga strain glycoprotein was performed as previously described[151].

7.2.2.63. Neutralisation assays

The whole Ebola virus neutralization assay[232] and the pseudotyped lentivirus neutralisation assay were performed as described previously [233].

The Ebola virus (EBOV) competition ELISA is based on the one-step incubation simultaneous competitive principle. If present in the sample, anti-EBOV antibodies compete with monoclonal anti-EBOV antibodies (Mab) conjugated to horseradish peroxide (HRP) for a fixed amount of recombinant EBOV GP pre-coated in the wells. When no anti-EBOV antibodies are present in the sample, the HRP labelled anti-EBOV binds to the EBOV GP antigens. Binding is reduced proportionally with increasing amounts of EBOV antibody specific for the epitope recognised by the Mab in the test serum.

Purified Mab 4G7, shown to neutralize EBOV[234] was conjugated to horseradish peroxidase (HRP) by standard methods[235]. Diluted test sera/plasma and the 4G7-HRP conjugate were added simultaneously to microwells coated with rGPδTM (IBT

Bioservises Inc. USA) and competed for the rGP binding sites. Any serum/plasma or dilution thereof giving inhibition of 4G7-HRP binding >50% was considered to contain potential neutralising antibody. Calculation of percentage inhibition was as follows:

Optical density 450/620nm of (NC-PC) – T/(NC-PC) x100

where NC is the OD obtained for a control normal human plasma sample that does not contain EBOV antibodies, PC is the OD obtained for the a sample that inhibits the antibody-HRP conjugate and T is the OD obtained for the competing test sample.

7.2.2.64. T cell assays

Ex vivo ELISPOT and intracellular cytokine staining assays were performed largely as previously described[7,11] using overlapping peptide pools except that 200,000 freshly isolated PBMC were added to each well and the final concentration of each peptide in the well was 2.5 µg/ml. Peptides were 15mers in length overlapping by 11 amino acids sequences as described elsewhere[2]. Peptides were custom synthesized at a minimum purity of 80% with a free amine and a free acid at the N and C terminus respectively, with HPLC chromatograms to confirm purity and mass spectral analysis to confirm identity (NeoBiolabs, Cambridge, MA). Pools of peptides were prepared according to the structural elements of the glycoprotein consisting of a single pool of peptides corresponding to the signal peptide (aa 1-32), 7 pools comprised of peptides from the glycoprotein chain 1 (aa 33-501) and 2 pools comprised of peptides from the glycoprotein chain 2 (aa 502-676). Responses were averaged across triplicate wells, and then responses in unstimulated (negative control) wells were subtracted. Responses to the negative control were always less than 84 spot-

forming cells per million PBMC (SFC) and the lower limit of detection for the ELISPOT assay was 50 SFC. Responses were considered positive if they were more than twice the corresponding negative control and greater than the median plus three standard deviations of the negative controls for all samples in the study, which equates to 41 SFC.

7.2.2.65. Flow cytometry

Intracellular cytokine staining was performed as previously described[1]. Freshly isolated PBMC were stimulated with a pool containing all 187 peptides spanning the glycoprotein at 2.5µg/ml. Samples were analysed on a LSR II cytometer (Becton Dickinson, Oxford, UK) and a minimum of 1 million events were collected for each sample. At least 83,000 live $CD3^+$ cells were analysed per sample. Data were prepared and analysed using FlowJo v9.8.1 (Treestar Inc., Ashland, Oregon, USA), Pestle v1.6 and Spice v5.05 (Mario Roederer, Vaccine Research Centre, NIAID, NIH). A hierarchical gating strategy was used. Responses to peptide were determined after subtraction of the response in the unstimulated control for each sample. Pie-charts were created using absolute measures with a threshold of 0.004%. For analyses of multiple cytokine function, all samples had >24,000 $CD4^+$ or $CD8^+$ T cells in the parent population. Samples where a response to the positive control of greater than 1% cytokine positive $CD4^+$ or $CD8^+$ T cells could not be detected were excluded from the analysis. The lower limit of detection for the assay was 0.004% and a positive response was greater than 2 times the medium control for the corresponding sample. One volunteer in group 3 (042) was excluded from the analysis of T cell responses due to high pre-existing responses to the glycoprotein peptides.

7.2.2.66. Statistical Analysis

Data was analyzed using a variety of software including Microsoft Excel for Windows version 14 (Microsoft Corporation, Washington, USA), SPSS for Mac version 23.0 (IBM Corp., USA) and GraphPad Prism for Windows or for Mac version 6 (GraphPad Software Inc., California, USA). All tests were 2-tailed.

7.3. Safety Appendix

7.3.1. VAC055: Solicited Adverse Events

A: Group 1 (n=20)

	Mild (%)	Mod (%)	Sev (%)
Pain	10 (50.0)	7 (35.0)	1 (5.0)
Warmth	9 (45.0)	1 (5.0)	0 (0.0)
Swelling	2 (10.0)	1 (5.0)	0 (0.0)
Redness	3 (15.0)	0 (0.0)	0 (0.0)
Itch	0 (0.0)	0 (0.0)	0 (0.0)
Fever	5 (25.0)	1 (5.0)	0 (0.0)
Feverishness	4 (20.0)	5 (25.0)	0 (0.0)
Myalgia	9 (45.0)	2 (10.0)	0 (0.0)
Arthralgia	7 (35.0)	0 (0.0)	0 (0.0)
Headache	11 (55.0)	2 (10.0)	0 (0.0)
Fatigue	8 (40.0)	2 (10.0)	0 (0.0)
Nausea	3 (15.0)	2 (10.0)	0 (0.0)
Malaise	5 (25.0)	2 (10.0)	0 (0.0)

B: Group 2 (n=17)

	Mild (%)	Mod (%)	Sev (%)
Pain	10 (58.8)	4 (23.5)	1 (5.9)
Warmth	11 (64.7)	1 (5.9)	0 (0.0)
Swelling	2 (11.8)	1 (5.9)	0 (0.0)
Redness	2 (11.8)	0 (0.0)	0 (0.0)
Itch	1 (5.9)	0 (0.0)	0 (0.0)
Fever	1 (5.9)	0 (0.0)	1 (5.9)
Feverishness	4 (23.5)	3 (17.6)	1 (5.9)
Myalgia	2 (11.8)	3 (17.6)	1 (5.9)
Arthralgia	2 (11.8)	4 (23.5)	0 (0.0)
Headache	4 (23.5)	0 (0.0)	1 (5.9)
Fatigue	2 (11.8)	5 (29.4)	1 (5.9)
Nausea	1 (5.9)	0 (0.0)	0 (0.0)
Malaise	4 (23.5)	1 (5.9)	1 (5.9)

C: Group 1 (n=20)

	Mild (%)	Mod (%)	Sev (%)
Pain	16 (80.0)	3 (15.0)	1 (5.0)
Warmth	12 (60.0)	1 (5.0)	0 (0.0)
Swelling	1 (5.0)	0 (0.0)	3 (15.0)
Redness	0 (0.0)	0 (0.0)	0 (0.0)
Itch	2 (10.0)	0 (0.0)	0 (0.0)
Fever	2 (10.0)	3 (15.0)	0 (0.0)
Feverishness	5 (25.0)	7 (35.0)	2 (10.0)
Myalgia	8 (40.0)	3 (15.0)	0 (0.0)
Arthralgia	4 (20.0)	1 (5.0)	0 (0.0)
Headache	10 (50.0)	6 (30.0)	1 (5.0)
Fatigue	11 (55.0)	2 (10.0)	0 (0.0)
Nausea	4 (20.0)	0 (0.0)	0 (0.0)
Malaise	5 (25.0)	2 (10.0)	0 (0.0)

D: Group 2 (n=17)

	Mild (%)	Mod (%)	Sev (%)
Pain	11 (64.7)	4 (23.5)	1 (5.9)
Warmth	10 (58.8)	3 (17.6)	0 (0.0)
Swelling	1 (5.9)	2 (11.8)	2 (11.8)
Redness	0 (0.0)	0 (0.0)	0 (0.0)
Itch	4 (23.5)	0 (0.0)	0 (0.0)
Fever	2 (11.8)	3 (17.6)	1 (5.9)
Feverishness	5 (29.4)	2 (11.8)	3 (17.6)
Myalgia	5 (29.4)	4 (23.5)	0 (0.0)
Arthralgia	6 (35.3)	3 (17.6)	0 (0.0)
Headache	5 (29.4)	3 (17.6)	0 (0.0)
Fatigue	4 (23.5)	4 (23.5)	2 (11.8)
Nausea	3 (17.6)	2 (11.8)	0 (0.0)
Malaise	2 (11.8)	5 (29.4)	2 (11.8)

E: Group 1 (n=18)

	Mild (%)	Mod (%)	Sev (%)
Pain	12 (66.7)	5 (27.8)	0 (0.0)
Warmth	9 (50.0)	1 (5.6)	0 (0.0)
Swelling	1 (5.6)	0 (0.0)	2 (11.1)
Redness	3 (16.7)	1 (5.6)	0 (0.0)
Itch	2 (11.1)	0 (0.0)	0 (0.0)
Fever	3 (16.7)	3 (16.7)	1 (5.6)
Feverishness	4 (22.2)	9 (50.0)	2 (11.1)
Myalgia	8 (44.4)	4 (22.2)	0 (0.0)
Arthralgia	4 (22.2)	3 (16.7)	1 (5.6)
Headache	11 (61.1)	3 (16.7)	1 (5.6)
Fatigue	9 (50.0)	3 (16.7)	1 (5.6)
Nausea	3 (16.7)	1 (5.6)	1 (5.6)
Malaise	5 (27.8)	3 (16.7)	0 (0.0)

F: Group 2 (n=16)

	Mild (%)	Mod (%)	Sev (%)
Pain	12 (75.0)	1 (6.3)	0 (0.0)
Warmth	11 (68.8)	0 (0.0)	0 (0.0)
Swelling	4 (25.0)	1 (6.3)	1 (6.3)
Redness	3 (18.8)	0 (0.0)	0 (0.0)
Itch	1 (6.3)	0 (0.0)	0 (0.0)
Fever	2 (12.5)	3 (18.8)	0 (0.0)
Feverishness	5 (31.3)	3 (18.8)	2 (12.5)
Myalgia	5 (31.3)	2 (12.5)	0 (0.0)
Arthralgia	3 (18.8)	1 (6.3)	0 (0.0)
Headache	4 (25.0)	7 (43.8)	1 (6.3)
Fatigue	6 (37.5)	3 (18.8)	0 (0.0)
Nausea	3 (18.8)	1 (6.3)	0 (0.0)
Malaise	3 (18.8)	4 (25.0)	1 (6.3)

G: ChAd63 ME-TRAP (n=20)

	Mild (%)	Mod (%)	Sev (%)
Pain	9 (45.0)	2 (10.0)	0 (0.0)
Warmth	6 (30.0)	0 (0.0)	0 (0.0)
Swelling	1 (5.0)	1 (5.0)	0 (0.0)
Redness	0 (0.0)	1 (5.0)	0 (0.0)
Itch	0 (0.0)	0 (0.0)	0 (0.0)
Fever	6 (30.0)	0 (0.0)	0 (0.0)
Feverishness	4 (20.0)	6 (30.0)	0 (0.0)
Myalgia	5 (25.0)	3 (15.0)	0 (0.0)
Arthralgia	5 (25.0)	1 (5.0)	0 (0.0)
Headache	6 (30.0)	5 (25.0)	0 (0.0)
Fatigue	8 (40.0)	2 (10.0)	0 (0.0)
Nausea	2 (10.0)	1 (5.0)	0 (0.0)
Malaise	3 (15.0)	2 (10.0)	0 (0.0)

H: MVA ME-TRAP (n=17)

	Mild (%)	Mod (%)	Sev (%)
Pain	8 (47.1)	8 (47.1)	1 (5.9)
Warmth	8 (47.1)	0 (0.0)	0 (0.0)
Swelling	2 (11.8)	0 (0.0)	0 (0.0)
Redness	5 (29.4)	0 (0.0)	0 (0.0)
Itch	1 (5.9)	0 (0.0)	0 (0.0)
Fever	6 (35.3)	2 (11.8)	0 (0.0)
Feverishness	9 (52.9)	3 (17.6)	3 (17.6)
Myalgia	6 (35.3)	2 (11.8)	1 (5.9)
Arthralgia	7 (41.2)	2 (11.8)	0 (0.0)
Headache	11 (64.7)	2 (11.8)	0 (0.0)
Fatigue	12 (70.6)	2 (11.8)	1 (5.9)
Nausea	5 (29.4)	0 (0.0)	0 (0.0)
Malaise	5 (29.4)	0 (0.0)	0 (0.0)

Table 56: VAC055. A & B: Solicited adverse events reported in the 7 day period following vaccination with dose 1 of RTS,S/AS01B in A: Group 1 subjects; B: Group 2 subjects; C & D: Solicited adverse events reported in the 7 day period following vaccination with dose 2 of RTS,S/AS01B in C: Group 1 subjects; D: Group 2 subjects; E & F: Solicited adverse events reported in the 7 day period following vaccination with dose 3 of RTS,S/AS01B in E: Group 1 subjects; F: Group 2 subjects G & H: Solicited adverse events reported by Group 1 subjects in G: the 7 day period following vaccination with ChAd63 ME-TRAP; H: the 7 day period following vaccination with MVA ME-TRAP

7.3.2. VAC055 Unsolicited Adverse Events

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Abdominal cramps	10000057	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Agitated	10001495	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Allergic rhinitis	10001723	2 (10.0)	0 (0.0)	0 (0.0)	2 (10.0)	2	0	0	2
Axillary pain	10048750	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Backache	10003993	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Bloated feeling	10005264	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Chills	10008531	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Colposcopy	10010069	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Coryzal symptoms	10011216	2 (10.0)	0 (0.0)	1 (5.0)	3 (15.0)	2	0	1	3
Cough	10011224	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	1	1	0	2
Dizziness	10013573	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Dry skin	10013786	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Feeling hot	10016334	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Gastroenteritis	10017888	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	0	0	1	1
Heartburn	10019326	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Hunger	10020466	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Injection site bruising	10022052	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Itchy scalp	10023093	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Light headed feeling	10024491	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Localized erythema	10024784	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Localized superficial swelling, mass or lump	10024784	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Nasal congestion	10028735	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Pain in elbow	10033424	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Period pains	10034532	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	1	1	0	2
Pharyngitis	10034835	3 (15.0)	0 (0.0)	0 (0.0)	3 (15.0)	3	0	0	3
Sensation of warmth	10040006	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Sneezing	10041232	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	2	1	0	3
Subjective visual disturbance, unspecified	10042399	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Thumb sprain	10043659	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Tight chest	10043854	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Toothache	10044057	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Vomiting	10047700	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	0	0	1	1

Table 57: VAC055. Frequency and severity of unsolicited AEs reported by Group 1 subjects in the 30 day period following vaccination with dose 1 of RTS,S/AS01B. Proportion is performed on the per protocol cohort (n=20)

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Abdominal pain	10000081	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Blisters	10005216	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Chest pain	10008479	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Chills	10008531	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Coryzal symptoms	10011216	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Cough	10011224	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Diarrhoea	10012735	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Dislocation of shoulder	10013182	0 (0.0)	0 (0.0)	1 (5.9)	1 (5.9)	0	0	1	1
Exacerbation of asthma	10015575	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Insomnia	10022437	0 (0.0)	2 (11.8)	0 (0.0)	2 (11.8)	0	2	0	2
Knee injury	10049032	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Light headedness	10024461	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Nasal congestion	10028735	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Paraesthesia foot	10052407	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Pharyngitis	10034835	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Rectal bleeding	10038035	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Rhinorrhoea	10039101	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Sensation of heaviness	10040000	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Urinary frequency	10046539	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Wasp sting	10047831	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1

Table 58: VAC055. Frequency and severity of unsolicited AEs reported by Group 2 subjects in the 30 day period following vaccination with dose 1 of RTS,S/AS01B. Proportion is performed on the per protocol cohort (n=17)

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Abdominal cramps	10000057	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Agitated	10001495	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Allergic rhinitis	10001723	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Chills	10008531	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Coryzal symptoms	10011216	3 (15.0)	0 (0.0)	1 (5.0)	4 (20.0)	3	0	1	4
Cough	10011224	2 (10.0)	1 (5.0)	0 (0.0)	3 (15.0)	2	1	0	3
Diarrhoea	10012735	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Dizziness	10013573	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Dry cough	10013773	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Dry skin	10013786	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Feeling hot	10016334	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Gastroenteritis	10017888	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	0	0	1	1
Heartburn	10019326	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Hunger	10020466	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Injection site bruising	10022052	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Insomnia	10022437	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Laryngitis	10023874	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Light headed feeling	10024491	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Localized erythema	10024782	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Localized superficial swelling, mass or lump	10024784	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Lymphadenopathy cervical	10025200	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Middle ear effusion	10062545	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Muscle cramp	10028294	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	0	0	1	1
Nasal congestion	10028735	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Period pains	10034532	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Pharyngitis	10034835	3 (15.0)	0 (0.0)	0 (0.0)	3 (15.0)	3	0	0	3
Red throat	10038196	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Sensation of warmth	10040006	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	1	1	0	2
Sneezing	10041232	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	2	1	0	3
Subjective visual disturbance,	10042399	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1

unspecified									
Thumb sprain	10043659	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Tight chest	10043854	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Toothache	10044057	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1

Table 59: VAC055. Frequency and severity of unsolicited AEs reported by Group 1 subjects in the 30 day period following vaccination with ChAd63 ME-TRAP. Proportion is performed on the per protocol cohort (n=17)

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Agitated	10001495	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Chills	10008531	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Coryzal symptoms	10011216	3 (15.0)	1 (5.0)	0 (0.0)	4 (20.0)	4	1	0	5
Cough	10011224	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	1	1	0	2
Diarrhoea	10012735	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Dizziness	10013573	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Dry cough	10013773	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Dry skin	10013786	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Feeling hot	10016334	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Heartburn	10019326	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Indigestion	10021706	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Insomnia	10022437	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Knee pain	10023477	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Laryngitis	10023874	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Localized erythema	10024782	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	2	0	0	2
Loose bowels	10024838	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Low back pain	10024891	1 (5.0)	0 (0.0)	1 (5.0)	2 (10.0)	1	0	1	2
Lymphadenopathy cervical	10025200	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Middle ear effusion	10062545	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Muscle cramp	10028294	0 (0.0)	0 (0.0)	1 (5.0)	1 (5.0)	0	0	1	1
Nasal congestion	10028735	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Neck pain	10028836	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Night sweats	10029410	2 (10.0)	0 (0.0)	0 (0.0)	2 (10.0)	2	0	0	2
Pharyngitis	10034835	2 (10.0)	0 (0.0)	0 (0.0)	2 (10.0)	2	0	0	2
Pulled hamstring	10037305	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Red throat	10038196	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Sensation of warmth	10040006	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	1	1	0	2
Sneezing	10041232	1 (5.0)	1 (5.0)	0 (0.0)	2 (10.0)	1	1	0	2

Subjective visual disturbance, unspecified	10042399	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Swelling, mass or lump in head and neck	10042710	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)	1	0	0	1
Tight chest	10043854	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1
Toothache	10044055	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	0	1	0	1

Table 60: VAC055. Frequency and severity of unsolicited AEs reported by Group 1 subjects in the 30 day period following vaccination with dose 2 of RTS,S/AS01B. Proportion is performed on the per protocol cohort (n=20)

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Abdominal pain	10000081	2 (11.8)	0 (0.0)	0 (0.0)	2 (11.8)	3	0	0	3
Asthmatic wheezing	10049200	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Backache	10003993	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Chest pain	10008479	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Chills	10008531	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Constipation	10010774	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Coryzal symptoms	10011216	2 (11.8)	0 (0.0)	0 (0.0)	2 (11.8)	2	0	0	2
Cough	10011224	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Diarrhoea	10012735	0 (0.0)	2 (11.8)	0 (0.0)	2 (11.8)	0	2	0	2
Dislocation of shoulder	10013182	0 (0.0)	0 (0.0)	1 (5.9)	1 (5.9)	0	0	1	1
Eye pain	10015958	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Head injury	10019196	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Insomnia	10022437	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Knee pain	10023477	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Menstrual cycle shortened	10027326	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Migraine	10027599	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Night sweats	10029410	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Rectal bleeding	10038035	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Shooting pain	10050007	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Sore throat	10041367	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Subjective visual disturbance, unspecified	10042399	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Sweating	10042661	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1

Vomiting	10047700	1 (5.9)	1 (5.9)	0 (0.0)	2 (11.8)	1	1	0	2
Wasp sting	10047831	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Wrist pain	10048692	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1

Table 61: VAC055. Frequency and severity of unsolicited AEs reported by Group 2 subjects in the 30 day period following vaccination with dose 2 of RTS,S/AS01B. Proportion is performed on the per protocol cohort (n=17)

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Allergic skin reaction	10001729	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Backache	10003993	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Chills	10008531	1 (5.6)	1 (5.6)	0 (0.0)	2 (11.1)	1	1	0	2
Coryzal symptoms	10011216	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Eyelid rash	10074620	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Indigestion	10021706	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Insomnia	10022437	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1
Knee pain	10023477	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1
Light headedness	10024461	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Localized erythema	10024781	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Loose bowels	10024838	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1
Low back pain	10024891	2 (11.1)	0 (0.0)	0 (0.0)	2 (11.1)	2	0	0	2
Nasal congestion	10028735	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Neck pain	10028836	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1
Night sweats	10029410	2 (11.1)	0 (0.0)	0 (0.0)	2 (11.1)	2	0	0	2
Pain in arm	10033421	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)	0	0	1	1
Pharyngitis	10034835	2 (11.1)	0 (0.0)	0 (0.0)	2 (11.1)	2	0	0	2
Polydipsia	10036067	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Rash erythematous	10037855	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Sensation of cold	10039997	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1
Sore throat	10041367	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Sweating fever	10042666	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1
Swelling, mass or lump in head and neck	10042710	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)	1	0	0	1
Toothache	10044055	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)	0	1	0	1

Table 62: VAC055. Frequency and severity of unsolicited AEs reported by Group 1 subjects in the 30 day period following vaccination with dose 3 of RTS,S/AS01B. Proportion is performed on the per protocol cohort (n=18)

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Abdominal pain	10000081	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Backache	10003993	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Coryzal symptoms	10011216	2 (12.5)	0 (0.0)	0 (0.0)	2 (12.5)	2	0	0	2
Cough	10011224	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Eye pain	10015958	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Head injury	10019196	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Local swelling	10024770	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Night sweats	10029410	0 (0.0)	1 (6.3)	0 (0.0)	1 (6.3)	0	1	0	1
Period pains	10034532	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Rash over arms	10037875	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Subjective visual disturbance, unspecified	10042399	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1
Sweating	10042661	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	1	0	0	1

Table 63: VAC055. Frequency and severity of unsolicited AEs reported by Group 2 subjects in the 30 day period following vaccination with dose 3 of RTS,S/AS01B. Proportion

MedDRA Preferred Term (PT)	MedDRA Code (PT)	Number of volunteers				Number of occurrences			
		Mild (%)	Mod (%)	Sev (%)	Total (%)	Mild	Mod	Sev	Total
Abdominal cramps	10000057	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Allergic skin reaction	10001729	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Athlete's foot	10003621	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Backache	10003993	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Buttock pain	10048677	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Chills	10008531	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Coryzal symptoms	10011216	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Cough	10011224	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Dizziness	10013573	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Epistaxis	10015090	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Eye pain	10015958	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Eyelid rash	10074620	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Head injury	10019196	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Insect bite NOS	10022403	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Insomnia	10022437	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Itching both hands	10023087	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Light headedness	10024461	1 (5.9)	1 (5.9)	0 (0.0)	2 (11.8)	1	1	0	2
Low back pain	10024891	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Nasal congestion	10028735	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1

Neck pain	10028836	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Pain in ankle	10033420	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Pain in leg	10033446	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Pain in thigh	10048973	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Palpitations	10033557	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Period pains	10034532	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Pharyngitis	10034835	1 (5.9)	1 (5.9)	0 (0.0)	2 (11.8)	2	1	0	3
Polydipsia	10036067	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Pruritis	10037086	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Rash erythematous	10037855	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Rhinorrhoea	10039101	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Sensation of cold	10039997	0 (0.0)	1 (5.9)	0 (0.0)	1 (5.9)	0	1	0	1
Sensation of warmth	10040006	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Toe injury	10051023	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Toothache	10044055	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Twitching	10045198	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1
Urinary frequency	10046539	1 (5.9)	0 (0.0)	0 (0.0)	1 (5.9)	1	0	0	1

Table 64: VAC055. Frequency and severity of unsolicited AEs reported by Group 1 subjects in the 30 day period following vaccination with MVA ME-TRAP. Proportion is performed on the per protocol cohort (n=16)

7.3.3. VAC055 Laboratory Adverse Events

Instance	Group	AE	Grade	Timepoints					
1	1	Anaemia	1	63	70				
2	2	Anaemia	1	7	63	70	83		
3	2	Anaemia	1	7					
1	1	Leukopenia	1	21	35	56	77		
1	1	Leukocytosis	1	35	63	70			
1	1	Thrombocytopenia	1	21					
1	1	Neutropenia	1	77					
2	1	Neutropenia	1	7	21	28	35	56	83
3	1	Neutropenia	2	77					
1	1	Lymphopenia	1	21					
1	1	Eosinophilia	1	35	56	63	70		
2	2	Eosinophilia	1	35					
3	1	Eosinophilia	1	7	21	35			
1	1	Hyponatraemia	1	77	83				
2	1	Hyponatraemia	1	77					
1	2	Hypokalaemia	1	56	70				
2	1	Hypokalaemia	1	7	56				
3	1	Hypokalaemia	1	70					
1	1	Uraemia	1	70					
2	2	Uraemia	1	56					
3	2	Uraemia	1	63	70				
1	1	Raised creatinine	1	21					
1	1	Hyperbilirubinaemia	1	21	35	63	70	77	83
2	2	Hyperbilirubinaemia	1	63					
3	1	Hyperbilirubinaemia	1	83					
4	2	Hyperbilirubinaemia	1	56					
5	1	Hyperbilirubinaemia	1	7	70				
6	1	Hyperbilirubinaemia	1	7	35				
7	2	Hyperbilirubinaemia	1	63					
8	2	Hyperbilirubinaemia	1	63	70				
9	1	Hyperbilirubinaemia	1	7	56	83			
10	1	Hyperbilirubinaemia	1	83					
1	1	Raised ALT	1	56	63	65	83		
1	2	Hypoalbuminaemia	1	35					
2	1	Hypoalbuminaemia	3	56					

Table 65: VAC055. All abnormal laboratory results within the adverse event range in the pre-CHMI period by severity and timepoint. Each line represents all instances at that severity in a single subject. See supplementary tables in appendix for severity grading criteria by study site. (Grade1 = mild; grade 2 = moderate; grade 3 = severe).

Instance	Group	AE	Grade	Timepoints							
1	2	Anaemia	1	C+35							
2	1	Anaemia	1	C+9	C+21	C+35	C+90	R+9	Rdiagnosis	RC+35	RC+90
3	1	Anaemia	1	C+9							
4	1	Anaemia	1	C+9	Diagnosis	C+35	RC-1	RC+35	RC+9	Rdiagnosis	
5	1	Anaemia	1	C+90							
6	2	Anaemia	1	C+9	C+35	C+90					
7	1	Anaemia	1	Diagnosis	Rdiagnosis	RC+35					
8	1	Anaemia	1	C+9							
1	2	Leukopenia	1	RC+13							
2	3	Leukopenia	1	c+9							
3	1	Leukopenia	1	Rdiagnosis							
4	1	Leukopenia	1	RDiagnosis							
1	2	Leukocytosis	1	C+90	RC+9						
1	3	Thrombocytopenia	1	Diagnosis							
2	2	Thrombocytopenia	2	RC+13							
1	1	Neutropenia	1	C+9	Diagnosis	RC-1	RC+9	Rdiagnosis			
2	3	Neutropenia	2	C+9							
3	4	Neutropenia	1	RC+35							
4	2	Neutropenia	2	RC+13							
1	3	Lymphopenia	1	Diagnosis							
2	2	Lymphopenia	1	C+90							
3	3	Lymphopenia	1	C+9	C+9						
4	1	Lymphopenia	1	Diagnosis							
5	1	Lymphopenia	2	Diagnosis							
6	3	Lymphopenia	2	Diagnosis							
7	1	Lymphopenia	2	EP+1							
8	2	Lymphopenia	2	Diagnosis							
9	3	Lymphopenia	3	Diagnosis							
10	2	Lymphopenia	3	C+14							
11	2	Lymphopenia	1	RDiagnosis							
12	4	Lymphopenia	1	RDiagnosis							
13	4	Lymphopenia	1	RDiagnosis							
14	4	Lymphopenia	3	RDiagnosis							
15	2	Lymphopenia	3	RC-1							
1	1	Eosinophilia	1	C+35							
2	4	Eosinophilia	1	RC+35							
3	1	Eosinophilia	1	RC-1	RC+6	RC+9					
4	4	Eosinophilia	2	RC+9							
1	4	Hypernatraemia	1	RC-1	RC+9						
2	4	Hypernatraemia	1	RC+90							
3	2	Hypernatraemia	1	RC-1							
4	2	Hyponatraemia	1	RC-1							
1	4	Hypokalaemia	1	RC+35							
2	4	Hypokalaemia	1	RC+35							

3	2	Hypokalaemia	1	Diagnosis							
4	2	Hypokalaemia	1	Diagnosis							
5	3	Hypokalaemia	2	C+90							
6	2	Hypokalaemia	2	Diagnosis							
7	3	Hypokalaemia	2	C+90							
1	1	Uraemia	1	C+35	C+90	RC+35					
2	1	Uraemia	1	C+9							
3	2	Uraemia	1	RC+35							
1	1	Hyperbilirubinaemia	1	C+90							
2	1	Hyperbilirubinaemia	1	C+9							
3	3	Hyperbilirubinaemia	1	C+35							
4	1	Hyperbilirubinaemia	2	C+35	C+90						
5	1	Hyperbilirubinaemia	2	Diagnosis							
6	1	Hyperbilirubinaemia	3	C+9	C+21						
7	2	Hyperbilirubinaemia	1	Re-screen							
8	2	Hyperbilirubinaemia	1	RC-1	RC+35	RC+90					
9	2	Hyperbilirubinaemia	1	RC+35							
1	1	Raised ALT	1	C+9							
2	2	Raised ALT	2	C+35							
3	3	Raised ALT	2	C+18							
4	1	Raised ALT	3	C+90	C+93						
1	1	Raised Alk Phos	1	C+18							

Table 66: VAC055. All abnormal laboratory results within the adverse event range in the post-CHMI period by severity and timepoint. Each line represents all instances at that severity in a single subject. See supplementary tables in appendix for severity grading criteria by study

7.3.4. VAC059 Solicited Adverse Events

A: Vaccine 1 : Group 1&2 (n=20)

	Mild (%)	Mod (%)	Sev (%)
Pain	11 (55)	7 (35)	0
Warmth	5 (25)	0	0
Swelling	1 (5)	0	0
Redness	3 (15)	3 (15)	0
Itch	1 (5)	0	0
Fever	0	0	0
Feverishness	8 (40)	1 (5)	0
Myalgia	9 (45)	2 (10)	0
Arthralgia	0	1 (5)	0
Headache	7 (35)	2 (10)	0
Fatigue	10 (50)	1 (5)	1 (5)
Nausea	2 (10)	0	0
Malaise	6 (30)	0	0

Vaccine 1: Group 3&4 (n=21)

	Mild (%)	Mod (%)	Sev (%)
Pain	13 (61.9)	7 (33.3)	1 (4.8)
Warmth	6 (28.6)	4 (19)	0
Swelling	1 (4.8)	0	0
Redness	6 (28.6)	0	0
Itch	3 (14.3)	0	0
Fever	3 (14.3)	6 (28.6)	1 (4.8)
Feverishness	6 (28.6)	7 (33.3)	3 (14.3)
Myalgia	9 (42.9)	4 (19)	1 (4.8)
Arthralgia	10 (47.6)	1 (4.8)	1 (4.8)
Headache	8 (38.1)	4 (19)	2 (9.5)
Fatigue	12 (57.1)	3 (14.3)	1 (4.8)
Nausea	5 (23.8)	1 (4.8)	0
Malaise	10 (47.6)	4 (19)	1 (4.8)

B: Vaccine 2: Group 1&2 (n=20)

	Mild (%)	Mod (%)	Sev (%)
Pain	10 (50)	4 (20)	1 (5)
Warmth	7 (35)	1 (5)	0
Swelling	0	0	0
Redness	2 (10)	3 (15)	1 (5)
Itch	2 (10)	0	0
Fever	4 (20)	3 (15)	0
Feverishness	6 (30)	8 (40)	1 (5)
Myalgia	12 (60)	1 (5)	1 (5)
Arthralgia	3 (15)	3 (15)	1 (5)
Headache	7 (35)	4 (20)	1 (5)
Fatigue	7 (35)	6 (30)	2 (10)
Nausea	6 (30)	0	0
Malaise	7 (35)	5 (25)	3 (15)

Vaccine 2: Group 3&4 (n=20)

	Mild (%)	Mod (%)	Sev (%)
Pain	10 (50)	9 (45)	1 (5)
Warmth	7 (35)	5 (25)	1 (5)
Swelling	3 (15)	2 (10)	2 (10)
Redness	6 (30)	3 (15)	1 (5)
Itch	6 (30)	1 (5)	0
Fever	2 (10)	2 (10)	3 (15)
Feverishness	7 (35)	9 (45)	3 (15)
Myalgia	3 (15)	10 (50)	3 (15)
Arthralgia	1 (5)	8 (40)	1 (5)
Headache	10 (50)	7 (35)	0
Fatigue	6 (30)	9 (45)	3 (15)
Nausea	10 (50)	0	0
Malaise	8 (40)	7 (35)	2 (10)

C: Vaccine 3: Group 1 (n=9)

	Mild (%)	Mod (%)	Sev (%)
Pain	8 (88.9)	0	0
Warmth	4 (44.4)	1 (11.1)	0
Swelling	0	0	0
Redness	1 (11.1)	1 (11.1)	0
Itch	3 (33.3)	0	0
Fever	8 (88.9)	0	0
Feverishness	3 (33.3)	1 (11.1)	1 (11.1)
Myalgia	4 (44.4)	1 (11.1)	1 (11.1)
Arthralgia	3 (33.3)	1 (11.1)	0
Headache	4 (44.4)	0	0
Fatigue	4 (44.4)	2 (22.2)	0
Nausea	2 (22.2)	0	0
Malaise	3 (33.3)	2 (22.2)	1 (11.1)

Vaccine 3: Group 2 (n=10)

	Mild (%)	Mod (%)	Sev (%)
Pain	5 (50)	1 (10)	0
Warmth	3 (30)	0	0
Swelling	1 (10)	0	0
Redness	0	0	0
Itch	0	0	0
Fever	0	0	0
Feverishness	0	0	0
Myalgia	4 (40)	0	0
Arthralgia	2 (20)	0	0
Headache	3 (30)	0	0
Fatigue	5 (50)	0	0
Nausea	2 (20)	0	0
Malaise	3 (30	0	0

Vaccine 3: Group 3 (n=10)

	Mild (%)	Mod (%)	Sev (%)
Pain	6 (60)	3 (30)	0
Warmth	6 (60)	1 (10)	1 (10)
Swelling	1 (10)	0	1 (10)
Redness	4 (40)	2 (20)	0
Itch	5 (50)	0	0
Fever	1 (10)	1 (10)	0
Feverishness	2 (20)	5 (50)	0
Myalgia	6 (60)	2 (20)	1 (10)
Arthralgia	3 (30)	1 (10)	1 (10)
Headache	4 (40)	2 (20)	0
Fatigue	7 (70)	2 (20)	0
Nausea	3 (30)	0	0
Malaise	5 (50)	2 (20)	0

Vaccine 3: Group 4 (n=10)

	Mild (%)	Mod (%)	Sev (%)
Pain	5 (50)	2 (20)	0
Warmth	3 (30)	2 (20)	0
Swelling	0	2 (20)	1 (10)
Redness	5 (50)	0	0
Itch	1 (10)	0	0
Fever	1 (10)	0	0
Feverishness	3 (30)	2 (20)	0
Myalgia	4 (40)	1 (10)	0
Arthralgia	3 (30)	1 (10)	0
Headache	3 (30)	1 (10)	0
Fatigue	5 (50)	2 (20)	0
Nausea	0	1 (10)	0
Malaise	1 (10)	3 (30)	0

Table 67: VAC059. Solicited adverse events reported in the 7 day period following each vaccination

A: Vaccination 1. Group 1 & 2 subjects received a full dose of RTS,S/AS01$_B$ only; Group 3 & 4 received a full dose of RTS,S/AS01$_B$ with a concomitant dose of ChAd63 ME-TRAP B: Vaccination 2. Group 1 & 2 subjects received a full dose of RTS,S/AS01$_B$ only; Group 3 & 4 received a full dose of RTS,S/AS01$_B$ with a concomitant dose of MVA ME-TRAP. C: Vaccination 3. Group 1 subjects received a full dose of RTS,S/AS01$_B$ only; Group 2 subjects received a fractional dose of RTS,S/AS01$_B$ at 1/5th standard dose only; Group 3 received a full dose of RTS,S/AS01$_B$ with a

concomitant dose of MVA ME-TRAP; Group 4 received a fractional dose of RTS,S/AS01$_B$ at 1/5th standard dose with a concomitant dose of MVA ME-TRAP

7.3.5. VAC059 Unsolicited Adverse Events

A: Groups 1 & 2 Vaccine 1

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10003988	Back pain	0	0	1	Possible
10008531	Chills	0	1	1	Probable
10061428	Decreased appetite	0	0	1	Probable
10020568	Hyperaesthesia	1	1	1	Possible
10068319	Oropharyngeal pain	1	1	1	Possible
10000087	Abdominal pain upper	1	1	1	Possible
10018964	Haemoptysis	2	2	1	Possible
10000087	Abdominal pain upper	3	3	1	Possible
10068319	Oropharyngeal pain	6	7	1	Possible
10068319	Oropharyngeal pain	7	7	1	Unlikely
10016256	Fatigue	7	9	1	Possible
10068319	Oropharyngeal pain	9	9	1	Unlikely
10019211	Headache	9	10	1	Unlikely
10012735	Diarrhoea	10	10	2	Unlikely
10011224	Cough	10	11	2	Unlikely
10011224	Cough	13	15	1	Unlikely
10019211	Headache	13	14	1	Unlikely
10068319	Oropharyngeal pain	14	14	1	Unlikely
10028735	Nasal congestion	14	15	1	Unlikely
10068319	Oropharyngeal pain	15	17	1	Unlikely
10019211	Headache	13	15	2	Unlikely
10028813	Nausea	17	18	2	Unlikely
10019211	Headache	18	18	1	Unlikely
10068319	Oropharyngeal pain	17	20	2	Unlikely
10068319	Oropharyngeal pain	19	21	1	Unlikely
10011224	Cough	19	21	1	Unlikely
10019211	Headache	20	20	1	Unlikely
10013946	Dyspepsia	25	26	1	Unlikely
10028810	Nasopharyngitis	26	27	1	Unlikely
10019211	Headache	25	26	2	Unlikely
10019211	Headache	26	27	2	Unlikely
10028813	Nausea	27	27	2	Unlikely
10028411	Myalgia	27	27	2	Unlikely

B: Groups 1 & 2 Vaccine 2

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10008531	Chills	0	0	1	Probable
10043458	Thirst	1	1	3	Possible
10037844	Rash	1	1	1	Possible
10015150	Erythema	1	1	1	Probable
10008531	Chills	1	1	2	Probable
10010947	Coordination abnormal	2	2	1	Possible
10014020	Ear pain	2	2	1	Possible
10068319	Oropharyngeal pain	3	3	1	Possible
10011224	Cough	3	5	1	Possible
10037844	Rash	5	5	1	Possible
10009245	Clavicle fracture	6	6	1	Possible
10016256	Fatigue	8	10	2	Possible
10019211	Headache	8	10	2	Possible
10019211	Headache	8	9	1	Possible
10017553	Furuncle	11	20	1	Unlikely
10019211	Headache	12	12	1	Unlikely
10013946	Dyspepsia	13	13	1	Unlikely
10016256	Fatigue	13	13	2	Possible
10028813	Nausea	13	13	2	Unlikely
10003239	Arthralgia	13	15	1	Unrelated
10019211	Headache	14	14	1	Unlikely
10025482	Malaise	15	18	1	Possible
10011224	Cough	16	20	2	Unlikely
10024968	Lower respiratory tract infection	16	16	1	Unlikely
10019211	Headache	16	17	1	Unlikely
10024770	Local swelling	16	20	1	Unlikely
10028735	Nasal congestion	17	18	2	Unlikely
10067152	Oral herpes	17	19	1	Unlikely
10009245	Clavicle fracture	18	20	3	Unrelated
10012735	Diarrhoea	19	20	1	Unlikely
10029410	Night sweats	19	19	1	Possible
10041232	Sneezing	19	20	1	Unlikely
10000087	Abdominal pain upper	20	20	1	Unlikely
10019211	Headache	20	20	1	Unlikely
10039101	Rhinorrhoea	21	21	1	Unlikely
10036790	Productive cough	21	21	1	Unlikely

C: Group 1 Vaccine 3

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10012735	Diarrhoea	0	0	1	Possible
10011224	Cough	0	1	1	Possible
10039101	Rhinorrhoea	0	1	1	Possible
10016326	Feeling cold	1	1	2	Possible
10027599	Migraine	3	4	1	Possible
10012735	Diarrhoea	4	4	1	Possible
10013911	Dysgeusia	10	11	1	Unlikely
10012735	Diarrhoea	11	11	1	Unlikely
10028411	Myalgia	15	15	2	Unlikely
10061428	Decreased appetite	19	20	2	Unlikely
10028813	Nausea	19	19	1	Unlikely
10068319	Oropharyngeal pain	20	20	1	Unlikely
10016256	Fatigue	20	20	1	Unlikely
10012378	Depression	20	20	1	Unlikely
10046571	Urinary tract infection	20	23	1	Unlikely
10047700	Vomiting	23	24	3	Unlikely
10012735	Diarrhoea	24	24	3	Unlikely
10019211	Headache	24	24	2	Unlikely
10022004	Influenza like illness	24	24	1	Unlikely
10010741	Conjunctivitis	24	24	1	Unlikely
10016256	Fatigue	24	24	2	Unlikely
10025482	Malaise	25	25	1	Unlikely

D: Group 2 Vaccine 2

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10017553	Furuncle	0	27	1	Possible
10012735	Diarrhoea	0	1	1	Possible
10061428	Decreased appetite	1	4	1	Possible
10017553	Furuncle	0	5	1	Possible
10000087	Abdominal pain upper	5	5	1	Possible
10037844	Rash	6	25	1	Possible
10068319	Oropharyngeal pain	7	8	1	Unlikely
10019211	Headache	9	9	1	Unlikely
10067152	Oral herpes	11	15	1	Unrelated
10019211	Headache	11	11	1	Unlikely
10028810	Nasopharyngitis	13	18	2	Unlikely
10019211	Headache	13	14	1	Unlikely

10019211	Headache	13	13	1	Unlikely
10019211	Headache	20	20	1	Unlikely
10052136	Ear congestion	21	27	1	Unlikely
10019211	Headache	22	22	2	Unlikely
10028735	Nasal congestion	24	24	1	Unlikely
10019211	Headache	24	24	2	Unlikely
10003399	Arthropod bite	25	26	1	Unrelated

E: Groups 3 & 4 Vaccine 1

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10039101	Rhinorrhoea	0	0	2	Possible
10016326	Feeling cold	0	0	3	Possible
10033775	Paraesthesia	0	1	1	Possible
10016326	Feeling cold	0	0	2	Possible
10008531	Chills	0	0	1	Probable
10046306	Upper respiratory tract infection	0	0	1	Possible
10016256	Fatigue	0	0	1	Probable
	Chills	0	0	2	Probable
10008531	Chills	0	0	2	Probable
10008531	Chills	0	0	2	Probable
10034568	Peripheral coldness	0	0	1	Possible
10008531	Chills	1	1	1	Probable
10020642	Hyperhidrosis	1	1	2	Probable
10028411	Myalgia	1	1	1	Probable
10003239	Arthralgia	1	1	1	Possible
10013573	Dizziness	1	1	1	Possible
10008531	Chills	1	1	1	Probable
10016326	Feeling cold	1	1	1	Possible
10015958	Eye pain	1	1	1	Possible
10024770	Local swelling	2	5	3	Probable
10050584	Contusion	2	6	1	Probable
10025188	Lymphadenitis	2	5	2	Probable
10015150	Erythema	2	4	2	Probable
10013935	Dysmenorrhoea	2	2	1	Possible
10014020	Ear pain	2	2	1	Possible
10037844	Rash	5	8	1	Possible
10013946	Dyspepsia	6	6	2	Possible
10039083	Rhinitis	7	8	1	Unlikely
10068881	Vaccination site pruritus	7	7	1	Probable
10019211	Headache	7	7	2	Unlikely
10019211	Headache	7	7	1	Unlikely
10040753	Sinusitis	8	15	2	Unlikely
10016256	Fatigue	8	9	1	Possible

10013946	Dyspepsia	9	9	2	Unlikely
10052576	Pruritus generalised	11	12	2	Possible
10068319	Oropharyngeal pain	14	17	1	Unlikely
10011224	Cough	14	26	2	Unlikely
10019211	Headache	15	18	2	Unlikely
10028411	Myalgia	16	17	1	Possible
10016256	Fatigue	16	18	2	Possible
10025482	Malaise	16	16	1	Possible
10037660	Pyrexia	16	16	1	Possible
10068319	Oropharyngeal pain	16	16	1	Unlikely
10037660	Pyrexia	16	19	2	Possible
10019211	Headache	16	17	2	Unlikely
10028411	Myalgia	16	19	1	Possible
10028813	Nausea	16	17	2	Unlikely
10028836	Neck pain	17	18	1	Unlikely
10003239	Arthralgia	16	19	1	Unlikely
10028735	Nasal congestion	18	19	1	Unlikely
10019211	Headache	19	25	2	Unlikely
10069489	Vaccination site exfoliation	19	19	1	Possible
10046306	Upper respiratory tract infection	19	22	1	Unlikely
10028735	Nasal congestion	20	23	1	Unlikely
10019211	Headache	20	22	2	Unlikely
10068319	Oropharyngeal pain	23	26	1	Unlikely
10028735	Nasal congestion	23	23	1	Unlikely
10011224	Cough	23	27	1	Unlikely
10016256	Fatigue	23	24	1	Unlikely
10039083	Rhinitis	24	27	1	Unlikely
10039083	Rhinitis	25	27	1	Unlikely
10019211	Headache	25	25	1	Unlikely
10048865	Hypoacusis	25	26	1	Unlikely
10019211	Headache	25	25	1	Unlikely
10028735	Nasal congestion	25	27	1	Unlikely
10011224	Cough	25	27	1	Unlikely
10040753	Sinusitis	26	27	1	Unlikely
10013573	Dizziness	26	26	1	Unlikely
10019211	Headache	27	27	1	Unlikely
10016256	Fatigue	27	27	1	Unlikely

F: Groups 3 & 4 Vaccine 2

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10013573	Dizziness	0	0	1	Possible
10069620	Vaccination site swelling	0	0	1	Definite
10008531	Chills	0	0	1	Probable
10013578	Dizziness postural	0	1	2	Possible
10021113	Hypothermia	0	1	2	Possible
10040753	Sinusitis	0	8	1	Possible
10052136	Ear congestion	0	1	1	Possible
10008531	Chills	0	0	2	Probable
10013573	Dizziness	0	0	1	Possible
10008531	Chills	0	0	2	Probable
10008531	Chills	0	0	2	Probable
10022437	Insomnia	0	0	1	Possible
10029446	Nocturia	0	3	1	Possible
10003549	Asthenia	0	0	3	Probable
10008531	Chills	0	0	1	Probable
10008531	Chills	0	1	2	Probable
10008531	Chills	1	1	2	Probable
10036790	Productive cough	1	1	1	Possible
10008531	Chills	1	1	1	Probable
10011224	Cough	1	1	1	Possible
10012735	Diarrhoea	1	1	2	Possible
10040984	Sleep disorder	1	1	3	Probable
10008531	Chills	1	1	3	Probable
10047700	Vomiting	1	1	1	Possible
10043071	Tachycardia	1	1	1	Possible
10014020	Ear pain	2	6	2	Possible
10069484	Vaccination site bruising	2	6	1	Probable
10061428	Decreased appetite	2	6	1	Possible
10059133	Cluster headache	4	4	2	Possible
10068319	Oropharyngeal pain	4	7	1	Possible
10028735	Nasal congestion	5	7	2	Possible
10036790	Productive cough	6	7	1	Possible
10069484	Vaccination site bruising	6	6	1	Probable
10028836	Neck pain	6	20	2	Possible
10019211	Headache	7	7	1	Unlikely
10016256	Fatigue	7	7	1	Possible
10003239	Arthralgia	7	21	2	Unrelated
10028391	Musculoskeletal pain	7	14	2	Possible
10047700	Vomiting	9	9	3	Unlikely
10012735	Diarrhoea	9	9	3	Unlikely

10019211	Headache	10	10	1	Unlikely
10018870	Blood urine present	10	12	2	Unlikely
10028813	Nausea	10	11	1	Unlikely
10059133	Cluster headache	11	12	2	Unlikely
10037844	Rash	12	12	1	Possible
10017553	Furuncle	12	122	1	Unlikely
10016256	Fatigue	12	12	2	Possible
10019211	Headache	13	13	1	Unlikely
10039083	Rhinitis	14	15	1	Unlikely
10052140	Eye pruritus	16	16	1	Unlikely
10052904	Musculoskeletal stiffness	16	16	1	Unlikely
10020568	Hyperaesthesia	17	17	1	Unlikely
10074170	Candida infection	18	18	1	Unlikely
10047700	Vomiting	19	19	2	Unlikely
10028813	Nausea	19	20	2	Unlikely
10016256	Fatigue	19	20	2	Unlikely
10028391	Musculoskeletal pain	20	20	1	Unlikely

G: Group 3 Vaccine 3

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10003239	Arthralgia	0	6	2	Possible
10003988	Back pain	1	1	1	Possible
10013573	Dizziness	1	1	2	Possible
10061458	Feeling of body temperature change	1	1	1	Probable
10040880	Skin irritation	1	1	2	Possible
10008531	Chills	1	1	2	Probable
10000087	Abdominal pain upper	1	1	2	Possible
10037844	Rash	2	2	1	Possible
10015150	Erythema	2	4	2	Probable
10059133	Cluster headache	3	3	2	Possible
10015150	Erythema	3	3	1	Possible
10059133	Cluster headache	9	12	3	Unlikely
10033425	Pain in extremity	14	14	1	Possible
10016256	Fatigue	14	15	1	Possible
10059133	Cluster headache	17	17	3	Unlikely
10016256	Fatigue	19	19	1	Unlikely
10019211	Headache	19	19	2	Unlikely
10059133	Cluster headache	23	23	3	Unlikely
10019211	Headache	24	24	1	Unlikely
10028411	Myalgia	25	27	1	Unlikely
10028836	Neck pain	27	27	2	Unrelated
10019211	Headache	27	27	2	Unlikely

10003988	Back pain	27	27	1	Unrelated

H: Group 4 Vaccine 3

PT code	Meddra PT	Day of onset (relative to vaccination)	Last day reported (relative to vaccination)	Max Severity	Causality
10043521	Throat irritation	0	0	1	Possible
10016326	Feeling cold	0	0	1	Possible
10028836	Neck pain	0	8	1	Possible
10016322	Feeling abnormal	0	1	1	Possible
10029446	Nocturia	1	3	1	Possible
10033557	Palpitations	1	1	1	Possible
10069481	Vaccination site paraesthesia	1	1	1	Probable
10028810	Nasopharyngitis	2	7	2	Possible
10047700	Vomiting	2	2	2	Possible
10068319	Oropharyngeal pain	2	4	2	Possible
10068319	Oropharyngeal pain	6	6	1	Possible
10013573	Dizziness	7	7	1	Possible
10015150	Erythema	7	7	1	Possible
10036790	Productive cough	8	9	1	Unlikely
10015090	Epistaxis	8	8	1	Unlikely
10016256	Fatigue	9	9	1	Possible
10028810	Nasopharyngitis	9	12	1	Unlikely
10019211	Headache	10	10	1	Unlikely
10068319	Oropharyngeal pain	9	12	1	Unlikely
10016256	Fatigue	11	11	1	Possible
10028735	Nasal congestion	9	12	1	Unlikely
10028813	Nausea	13	13	1	Unlikely
10028411	Myalgia	17	17	1	Unlikely
10028391	Musculoskeletal pain	19	19	1	Unlikely
10068319	Oropharyngeal pain	19	25	1	Unlikely
10011224	Cough	21	27	1	Unlikely
10074170	Candida infection	25	27	2	Unlikely
10016256	Fatigue	26	26	1	Unlikely

Table 68: VAC059. Unsolicited adverse events reported in the 28 day period following each vaccination with Meddra preferred term (PT) and code, day of onset relative to vaccination, last day AE reported, maximum reported severity and causality assessment outcome of relationship to vaccination. Severity grading: 1=mild; 2=moderate; 3=severe. A: Vaccination 1. Group 1 & 2 subjects received a full dose of RTS,S/AS01$_B$ only. B: Vaccination 2. Group 1 & 2 subjects received a full dose of RTS,S/AS01$_B$ only C: Vaccination 3. Group 1 subjects received a full dose of RTS,S/AS01$_B$ only. D: Vaccination 3. Group 2 subjects received a fractional dose of RTS,S/AS01$_B$ at 1/5th standard

dose only. E: Vaccination 1. Group 3 & 4 received a full dose of RTS,S/AS01$_B$ with a concomitant dose of ChAd63 ME-TRAP. F: Vaccination 2. Group 3 & 4 received a full dose of RTS,S/AS01$_B$ with a concomitant dose of MVA ME-TRAP. G: Vaccination 3. Group 3 received a full dose of RTS,S/AS01$_B$ with a concomitant dose of MVA ME-TRAP. H: Vaccination 3. Group 4 received a fractional dose of RTS,S/AS01$_B$ at 1/5th standard dose with a concomitant dose of MVA ME-TRAP

7.3.6. EBL01 Unsolicited Adverse Events

Adverse Event	Severity	Group 1 (n=19)	Group 2 and 4-7 (n=51)	Group 3 (n=20)	All Groups (n=90)
Abdominal distension	Mild	0	1 (2.0%)	0	1 (1.1%)
Abdominal Pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Abdominal pain upper	Mild	1 (5.3%)	1 (2.0%)	1 (5.0%)	3 (3.3%)
Abdominal pain upper	Moderate	0	0	1 (5.0%)	1 (1.1%)
Alcohol intolerance	Mild	0	0	1 (5.0%)	1 (1.1%)
Apthous Stomatitis	Mild	0	1 (2.0%)	0	1 (1.1%)
Apthous Stomatitis	Moderate	0	1 (2.0%)	0	1 (1.1%)
Axillary pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Back Pain	Mild	1 (5.3%)	2 (3.9%)	0	3 (3.3%)
Balance Disorder	Mild	0	1 (2.0%)	0	1 (1.1%)
Chest discomfort	Mild	0	1 (2.0%)	0	1 (1.1%)
Chest pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Chills	Mild	1 (5.3%)	2 (3.9%)	0	3 (3.3%)
Contusion	Mild	1 (5.3%)	0	0	1 (1.1%)
Cough	Mild	1 (5.3%)	1 (2.0%)	2 (10.0%)	4 (4.4%)
Cough	Moderate	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Decreased appetite	Mild	1 (5.3%)	2 (3.9%)	0	3 (3.3%)
Diarrhoea	Mild	1 (5.3%)	2 (3.9%)	1 (5.0%)	4 (4.4%)
Diarrhoea	Moderate	0	1 (2.0%)	0	1 (1.1%)
Diastolic hypertension	Mild	1 (5.3%)	1 (2.0%)	1 (5.0%)	3 (3.3%)
Dizziness	Mild	1 (5.3%)	1 (2.0%)	2 (10.0%)	4 (4.4%)
Dry Eye	Mild	0	1 (2.0%)	0	1 (1.1%)
Dysgeusia	Mild	1 (5.3%)	0	0	1 (1.1%)
Dysmenorrhoea	Moderate	0	3 (5.9%)	0	3 (3.3%)
Dyspepsia	Mild	0	1 (2.0%)	0	1 (1.1%)
Dyspepsia	Moderate	0	1 (2.0%)	0	1 (1.1%)
Dyspnoea	Mild	0	1 (2.0%)	0	1 (1.1%)
Ear Pain	Mild	1 (5.3%)	1 (2.0%)	2 (10.0%)	4 (4,4%)
Erythema	Mild	0	0	1 (5.0%)	1 (1.1%)
Fatigue	Mild	1 (5.3%)	3 (5.9%)	1 (5.0%)	5 (5.6%)
Fatigue	Moderate	0	1 (2.0%)	0	1 (1.1%)
Gingival bleeding	Mild	1 (5.3%)	0	0	1 (1.1%)
Groin Pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Headache	Mild	3 (15.8%)	3 (5.9%)	1 (5.0%)	7 (7.8%)
Headache	Moderate	2 (10.5%)	2 (3.9%)	3 (15.0%)	7 (7.8%)
Headache	Severe	0	2 (3.9%)	0	2 (2.2%)
Hyperhidrosis	Mild	0	0	1 (5.0%)	1 (1.1%)
Injection site bruising	Mild	0	1 (2.0%)	0	1 (1.1%)

Adverse Event	Severity	Group 1 (n=19)	Group 2 and 4-7 (n=51)	Group 3 (n=20)	All Groups (n=90)
Injection site pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Insomnia	Mild	1 (5.3%)	0	0	1 (1.1%)
Insomnia	Moderate	0	1 (2.0%)	0	1 (1.1%)
Laryngitis	Moderate	0	1 (2.0%)	0	1 (1.1%)
Lethargy	Moderate	0	0	2 (10.0%)	2 (2.2%)
Ligament sprain	Mild	1 (5.3%)	0	0	1 (1.1%)
Lymphadenopathy	Mild	1 (5.3%)	3 (5.9%)	1 (5.0%)	5 (5.6%)
Menstruation irregular	Mild	0	0	1 (5.0%)	1 (1.1%)
Multiple injuries	Moderate	0	1 (2.0%)	0	1 (1.1%)
Muscle strain	Moderate	1 (5.3%)	0	0	1 (1.1%)
Muscle Tightness	Mild	0	1 (2.0%)	0	1 (1.1%)
Myalgia	Mild	0	2 (3.9%)	0	2 (2.2%)
Myalgia	Moderate	0	0	1 (5.0%)	1 (1.1%)
Nasal congestion	Moderate	0	1 (2.0%)	0	1 (1.1%)
Nasal discomfort	Mild	0	1 (2.0%)	0	1 (1.1%)
Nasopharyngitis	Mild	4 (21.1%)	3 (5.9%)	1 (5.0%)	8 (8.9%)
Nasopharyngitis	Moderate	1 (5.3%)	0	0	1 (1.1%)
Nausea	Mild	0	2 (3.9%)	0	2 (2.2%)
Nausea	Moderate	0	1 (2.0%)	2 (10.0%)	3 (3.3%)
Oedema Mucosal	Mild	0	1 (2.0%)	0	1 (1.1%)
Oral herpes	Mild	1 (5.3%)	1 (2.0%)	0	2 (2.2%)
Oropharyngeal pain	Mild	1 (5.3%)	4 (7.8%)	3 (15.0%)	8 (8.9%)
Oropharyngeal pain	Moderate	0	1 (2.0%)	2 (10.0%)	3 (3.3%)
Pain in extremity	Mild	0	1 (2.0%)	0	1 (1.1%)
Paraesthesia	Mild	2 (10.5%)	1 (2.0%)	0	3 (3.3%)
Pharyngeal ulceration	Mild	0	1 (2.0%)	0	1 (1.1%)
Productive cough	Mild	1 (5.3%)	0	0	1 (1.1%)
Pruritis	Mild	1 (5.3%)	0	0	1 (1.1%)
Pyrexia	Mild	0	0	1 (5.0%)	1 (1.1%)
Pyrexia	Moderate	0	1 (2.0%)	0	1 (1.1%)
Rhinitis	Mild	1 (5.3%)	2 (3.9%)	3 (15.0%)	6 (6.7%)
Rhinorrhoea	Mild	0	4 (7.8%)	0	4 (4.4%)
Rhinorrhoea	Moderate	0	1 (2.0%)	0	1 (1.1%)
Sinus headache	Mild	0	1 (2.0%)	0	1 (1.1%)
Sinus pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Sneezing	Mild	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Sneezing	Moderate	1 (5.3%)	1 (2.0%)	0	2 (2.2%)
Stress	Moderate	0	2 (3.9%)	0	2 (2.2%)
Systolic hypertension	Mild	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Systolic hypertension	Moderate	0	0	1 (5.0%)	1 (1.1%)
Tachycardia	Mild	0	1 (2.0%)	2 (10.0%)	3 (3.3%)

Adverse Event	Severity	Group 1 (n=19)	Group 2 and 4-7 (n=51)	Group 3 (n=20)	All Groups (n=90)
Tendonitis	Mild	0	0	1 (5.0%)	1 (1.1%)
Thermal burn	Moderate	0	0	1 (5.0%)	1 (1.1%)
Thirst	Moderate	0	0	1 (5.0%)	1 (1.1%)
Tinnitus	Mild	0	1 (2.0%)	0	1 (1.1%)
Torticollis	Mild	0	1 (2.0%)	0	1 (1.1%)
Vaccination site discolouration	Mild	0	1 (2.0%)	0	1 (1.1%)
Vaccination site pain	Mild	1 (5.3%)	0	1 (5.0%)	2 (2.2%)
Vomiting	Mild	0	1 (2.0%)	0	1 (1.1%)

Table 69: EBL01. All unsolicited AEs reported. Frequency of unsolicited Aes reported in the 28 days following vaccination with ChAd3-EBO-Z (or until vaccination with MVA-BN® Filo). Frequency is calculated as the number of subjects counted once at worst severity. Rows with all zero values are not shown. Aes have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Preferred Term level. Subjects received ChAd3-EBO Z at the following doses: Group 1 – $1x10^{10}$vp; Groups 2, 4, 5, 6 & 7 – $2.5x10^{10}$vp; Group 3 – $5x10^{10}$vp

Adverse Event	Severity	Group 1 (n=19)	Group 2 and 4-7 (n=51)	Group 3 (n=20)	All Groups (n=90)
Abdominal distension	Mild	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Abdominal Pain	Mild	0	2 (3.9%)	0	2 (2.2%)
Abdominal pain upper	Mild	1 (5.3%)	0	0	1 (1.1%)
Apthous Stomatitis	Moderate	0	1 (2.0%)	0	1 (1.1%)
Axillary pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Back Pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Balance Disorder	Mild	0	1 (2.0%)	0	1 (1.1%)
Chest discomfort	Mild	0	1 (2.0%)	0	1 (1.1%)
Chest pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Chills	Mild	0	2 (3.9%)	0	2 (2.2%)
Cough	Mild	1 (5.3%)	2 (3.9%)	2 (10.0%)	5 (5.6%)
Cough	Moderate	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Decreased appetite	Mild	1 (5.3%)	2 (3.9%)	0	3 (3.3%)
Diarrhoea	Mild	1 (5.3%)	1 (2.0%)	1 (5.0%)	3 (3.3%)
Diastolic hypertension	Mild	1 (5.3%)	1 (2.0%)	1 (5.0%)	3 (3.3%)
Dizziness	Mild	1 (5.3%)	0	1 (5.0%)	2 (2.2%)
Dry Eye	Mild	0	1 (2.0%)	0	1 (1.1%)
Dysgeusia	Mild	1 (5.3%)	0	0	1 (1.1%)
Dyspnoea	Mild	0	1 (2.0%)	0	1 (1.1%)

Ear Pain	Mild	1 (5.3%)	1 (2.0%)	1 (5.0%)	3 (3.3%)
Fatigue	Mild	0	3 (5.9%)	0	3 (3.3%)
Fatigue	Moderate	0	1 (2.0%)	0	1 (1.1%)
Gingival bleeding	Mild	1 (5.3%)	0	0	1 (1.1%)
Groin Pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Headache	Mild	0	2 (3.9%)	2 (10.0%)	4 (4.4%)
Headache	Moderate	2 (10.5%)	0	1 (5.0%)	3 (3.3%)
Headache	Severe	0	1 (2.0%)	0	1 (1.1%)
Hyperhidrosis	Mild	0	0	1 (5.0%)	1 (1.1%)
Injection site bruising	Mild	0	1 (2.0%)	0	1 (1.1%)
Injection site pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Insomnia	Moderate	0	1 (2.0%)	0	1 (1.1%)
Lymphadenopathy	Mild	1 (5.3%)	3 (5.9%)	1 (5.0%)	5 (5.6%)
Menstruation irregular	Mild	0	0	1 (5.0%)	1 (1.1%)
Muscle Tightness	Mild	0	1 (2.0%)	0	1 (1.1%)
Myalgia	Mild	0	2 (3.9%)	0	2 (2.2%)
Nasal congestion	Moderate	0	1 (2.0%)	0	1 (1.1%)
Nausea	Mild	0	1 (2.0%)	0	1 (1.1%)
Oedema Mucosal	Mild	0	1 (2.0%)	0	1 (1.1%)
Oropharyngeal pain	Mild	1 (5.3%)	3 (5.9%)	2 (10.0%)	6 (6.7%)
Oropharyngeal pain	Moderate	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Pain in extremity	Mild	0	1 (2.0%)	0	1 (1.1%)
Paraesthesia	Mild	2 (10.5%)	1 (2.0%)	0	3 (3.3%)
Pharyngeal ulceration	Mild	0	1 (2.0%)	0	1 (1.1%)
Pruritis	Mild	1 (5.3%)	0	0	1 (1.1%)
Pyrexia	Moderate	0	1 (2.0%)	0	1 (1.1%)
Rhinorrhoea	Mild	0	4 (7.8%)	0	4 (4.4%)
Rhinorrhoea	Moderate	0	1 (2.0%)	0	1 (1.1%)
Sinus headache	Mild	0	1 (2.0%)	0	1 (1.1%)
Sinus pain	Mild	0	1 (2.0%)	0	1 (1.1%)
Sneezing	Mild	0	1 (2.0%)	0	1 (1.1%)
Sneezing	Moderate	0	1 (2.0%)	0	1 (1.1%)
Systolic hypertension	Mild	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Tachycardia	Mild	0	1 (2.0%)	1 (5.0%)	2 (2.2%)
Thirst	Moderate	0	0	1 (5.0%)	1 (1.1%)
Tinnitus	Mild	0	1 (2.0%)	0	1 (1.1%)
Vaccination site discolouration	Mild	0	1 (2.0%)	0	1 (1.1%)
Vaccination site pain	Mild	1 (5.3%)	0	1 (5.0%)	2 (2.2%)

Table 70: EBL01. Related unsolicited AEs. Frequency of unsolicited AEs reported in the 28 days following vaccination with ChAd3-EBO Z (or until vaccination with MVA-BN® Filo) assessed as being possibly, probably or definitely related to vaccination. Causality has been assigned to all unsolicited AEs based on type of event and temporal relationship to vaccination. Causality gradings are:

unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination. Frequency is calculated as the number of subjects counted once at worst severity. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Preferred Term level. Subjects received ChAd3-EBO Z at the following doses: Group 1 – $1x10^{10}$vp; Groups 2, 4, 5, 6 & 7 – $2.5x10^{10}$vp; Group 3 – $5x10^{10}$vp

Adverse Event	Severity	Subgroup b (n=12)	Subgroup c, & Groups 4 & 5 (n=34)	Groups 6 & 7 (n=15)	All groups (n=61)
Abdominal discomfort	Mild	1 (8.3%)	0	0	1 (1.6%)
Abdominal pain	Moderate	0	1 (2.9%)	0	1 (1.6%)
Abdominal pain upper	Moderate	0	1 (2.9%)	0	1 (1.6%)
Abdominal pain upper	Mild	0	2 (5.9%)	0	2 (3.3%)
Aesthenia	Severe	0	0	1 (6.7%)	1 (1.6%)
Apthous stomatitis	Mild	0	2 (12.5%)	0	2 (3.3%)
Arthralgia	Moderate	0	1 (2.9%)	0	1 (1.6%)
Arthralgia	Severe	0	0	1 (6.7%)	1 (1.6%)
Back pain	Mild	0	1 (2.9%)	0	1 (1.6%)
Back pain	Moderate	0	1 (2.9%)	0	1 (1.6%)
Chest discomfort	Mild	2 (16.7%)	0	0	2 (3.3%)
Cough	Moderate	2 (16.7%)	1 (2.9%)	1 (6.7%)	4 (6.6%)
Cough	Mild	0	3 (8.8%)	2 (13.3%)	5 (8.2%)
Depressed mood	Moderate	0	1 (2.9%)	0	1 (1.6%)
Diarrhoea	Moderate	0	2 (5.9%)	0	2 (3.3%)
Diarrhoea	Mild	0	1 (2.9%)	0	1 (1.6%)
Diastolic hypertension	Moderate	1 (8.3%)	0	1 (6.7%)	2 (3.3%)
Diastolic hypertension	Mild	0	2 (5.9%)	0	2 (3.3%)
Dizziness	Mild	0	1 (2.9%)	0	1 (1.6%)
Dry skin	Mild	0	1 (2.9%)	0	1 (1.6%)
Dyspepsia	Mild	1 (8.3%)	1 (2.9%)	0	2 (3.3%)
Ear Pain	Mild	0	0	1 (6.7%)	1 (1.6%)
Epistaxis	Mild	0	1 (2.9%)	0	1 (1.6%)
Fatigue	Mild	1 (8.3%)	1 (2.9%)	0	2 (3.3%)
Fatigue	Moderate	0	0	1 (6.7%)	1 (1.6%)
Fever	Severe	0	0	1 (6.7%)	1 (1.6%)
Headache	Mild	2 (16.7%)	7 (20.6%)	2 (13.3%)	11 (18%)
Headache	Moderate	1 (8.3%)	2 (5.9%)	1 (6.7%)	4 (6.6%)
Headache	Severe	0	0	1 (6.7%)	1 (1.6%)
Injection site bruising	Mild	0	1 (2.9%)	0	1 (1.6%)
Injection site	Mild	0	1 (2.9%)	0	1 (1.6%)

erythema					
Injection site pain	Moderate	1 (8.3%)	0	0	1 (1.6%)
Insomnia	Moderate	0	1 (2.9%)	0	1 (1.6%)
Joint dislocation	Severe	0	1 (2.9%)	0	1 (1.6%)
Joint stiffness	Mild	1 (8.3%)	0	0	1 (1.6%)
Lymphadenopathy	Mild	0	1 (2.9%)	1 (6.7%)	2 (3.3%)
Malaise	Moderate	0	1 (2.9%)	0	1 (1.6%)
Migraine	Moderate	1 (8.3%)	0	0	1 (1.6%)
Muscle strain	Moderate	0	1 (2.9%)	0	1 (1.6%)
Myalgia	Mild	1 (8.3%)	0	0	1 (1.6%)
Myalgia	Severe	0	0	1 (6.7%)	1 (1.6%)
Nasal congestion	Mild	2 (16.7%)	2 (5.9%)	0	4 (6.6%)
Nasal congestion	Moderate	0	1 (2.9%)	0	1 (1.6%)
Nasopharyngitis	Moderate	1 (8.3%)	1 (2.9%)	0	2 (3.3%)
Nasopharyngitis	Mild	1 (8.3%)	2 (5.9%)	0	3 (4.9%)
Nausea	Moderate	0	1 (2.9%)	1 (6.7%)	1 (2.2%)
Nausea	Mild	0	2 (5.9%)	0	2 (3.3%)
Oral herpes	Mild	0	1 (2.9%)	0	1 (1.6%)
Oropharyngeal pain	Mild	2 (16.7%)	2 (5.9%)	2 (13.3%)	6 (9.8%)
Oropharyngeal pain	Moderate	1 (8.3%)	1 (2.9%)	0	2 (3.3%)
Pain in extremity	Mild	0	1 (2.9%)	0	1 (1.6%)
Pharyngeal Ulceration	Mild	0	0	1 (6.7%)	1 (1.6%)
Procedural pain	Moderate	0	1 (2.9%)	0	1 (1.6%)
Productive cough	Mild	0	1 (2.9%)	1 (6.7%)	2 (3.3%)
Renal pain	Mild	1 (8.3%)	0	0	1 (1.6%)
Rhinorrhoea	Mild	0	2 (5.9%)	2 (13.3%)	4 (6.6%)
Rhinorrhoea	Moderate	0	0	1 (6.7%)	1 (1.6%)
Sneezing	Mild	1 (8.3%)	1 (2.9%)	0	2 (3.3%)
Systolic hypertension	Mild	2 (16.7%)	2 (5.9%)	1 (6.7%)	5 (8.2%)
Tachycardia	Mild	0	2 (5.9%)	0	2 (3.3%)
Thirst	Mild	0	1 (2.9%)	0	1 (1.6%)
Throat irritation	Mild	0	1 (2.9%)	0	1 (1.6%)
Toothache	Mild	0	1 (2.9%)	0	1 (1.6%)
Urticaria	Moderate	0	0	1 (6.7%)	1 (1.6%)
Vaccination site bruising	Mild	0	1 (2.9%)	0	1 (1.6%)
Vaccination site erythema	Mild	0	1 (2.9%)	0	1 (1.6%)
Vaccination site swelling	Mild	0	2 (5.9%)	0	2 (3.3%)
Vomiting	Moderate	0	1 (2.9%)	0	1 (1.6%)
Vomiting	Severe	0	1 (2.9%)	0	1 (1.6%)

Table 71: EBL01. All unsolicited AEs reported. Frequency of unsolicited AEs reported in the 28 days following vaccination with MVA-BN® Filo. Frequency is calculated as the number of subjects

counted once at worst severity. Rows with all zero values are not shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Preferred Term level. Subjects received MVA-BN® Filo at the following doses: Subgroup b – $3x10^8$pfu; Subgroup c and Groups 4 & 5 - $1.5x10^8$ pfu; Groups 6 & 7 – $3x10^7$pfu

Adverse Event	Severity	Subgroup b (n=12)	Subgroup c, & Groups 4 & 5 (n=34)	Group 6 & 7 (n=15)	All groups (n=61)
Abdominal pain upper	Mild	0	1 (2.9%)	0	1 (1.6%)
Arthralgia	Moderate	0	1 (2.9%)	0	1 (1.6%)
Chest discomfort	Mild	2 (16.7%)	0	0	2 (3.3%)
Cough	Moderate	1 (8.3%)	0	1 (6.7%)	2 (3.3%)
Cough	Mild	0	3 (8.8%)	1 (6.7%)	4 (6.6%)
Depressed mood	Moderate	0	1 (2.9%)	0	1 (1.6%)
Diarrhoea	Moderate	0	1 (2.9%)	0	1 (1.6%)
Diarrhoea	Mild	0	1 (2.9%)	0	1 (1.6%)
Diastolic hypertension	Mild	0	1 (2.9%)	0	1 (1.6%)
Dizziness	Mild	0	1 (2.9%)	0	1 (1.6%)
Dry skin	Mild	0	1 (2.9%)	0	1 (1.6%)
Dyspepsia	Mild	1 (8.3%)	0	0	1 (1.6%)
Fatigue	Mild	1 (8.3%)	0	0	1 (1.6%)
Fatigue	Moderate	0	0	1 (6.7%)	1 (1.6%)
Headache	Mild	0	1 (2.9%)	0	1 (1.6%)
Headache	Moderate	0	0	1 (6.7%)	1 (1.6%)
Injection site bruising	Mild	0	1 (2.9%)	0	1 (1.6%)
Injection site erythema	Mild	0	1 (2.9%)	0	1 (1.6%)
Injection site pain	Moderate	1 (8.3%)	0	0	1 (1.6%)
Insomnia	Moderate	0	1 (2.9%)	0	1 (1.6%)
Joint stiffness	Mild	1 (8.3%)	0	0	1 (1.6%)
Lymphadenopathy	Mild	0	1 (2.9%)	0	1 (1.6%)
Nasal congestion	Mild	1 (8.3%)	2 (5.9%)	0	3 (4.9%)
Nasopharyngitis	Moderate	0	1 (2.9%)	0	1 (1.6%)
Nausea	Moderate	0	1 (2.9%)	0	1 (1.6%)
Oropharyngeal pain	Mild	1 (8.3%)	1 (2.9%)	2 (13.3%)	4 (6.6%)
Oropharyngeal pain	Moderate	1 (8.3%)	0	0	1 (1.6%)
Pain in extremity	Mild	0	1 (2.9%)	0	1 (1.6%)
Productive cough	Mild	0	1 (2.9%)	0	1 (1.6%)
Renal pain	Mild	1 (8.3%)	0	0	1 (1.6%)
Rhinorrhoea	Mild	0	2 (5.9%)	2 (13.3%)	4 (6.6%)
Rhinorrhoea	Moderate	0	0	1 (6.7%)	1 (1.6%)

Sneezing	Mild	1 (8.3%)	0	0	1 (1.6%)
Systolic hypertension	Mild	2 (16.7%)	0	0	2 (3.3%)
Tachycardia	Mild	0	2 (5.9%)	0	2 (3.3%)
Thirst	Mild	0	1 (2.9%)	0	1 (1.6%)
Urticaria	Moderate	0	0	1 (6.7%)	1 (1.6%)
Vaccination site bruising	Mild	0	1 (2.9%)	0	1 (1.6%)
Vaccination site erythema	Mild	0	1 (2.9%)	0	1 (1.6%)
Vaccination site swelling	Mild	0	2 (5.9%)	0	2 (3.3%)
Vomiting	Severe	0	1 (2.9%)	0	1 (1.6%)

Table 72: EBL01. Related unsolicited AEs. Frequency of unsolicited AEs reported in the 28 days following vaccination with MVA-BN® Filo assessed as being possibly, probably or definitely related to vaccination. Causality has been assigned to all unsolicited AEs based on type of event and temporal relationship to vaccination. Causality gradings are: unrelated; unlikely relationship to vaccination; possible relationship to vaccination; probable relationship to vaccination; definite relationship to vaccination. Frequency is calculated as the number of subjects counted once at worst severity. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Preferred Term level. Subjects received MVA-BN® Filo at the following doses: Subgroup b – $3x10^8$pfu; Subgroup c and Groups 4 & 5 - $1.5x10^8$ pfu; Groups 6 & 7 – $3x10^7$pfu

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