RIVER
Research In Viral Eradication of HIV Reservoirs

Imperial College London

MRC Clinical Trials Unit

UNIVERSITY OF OXFORD

UNIVERSITY OF CAMBRIDGE

UCL

MRC Medical Research Council

RIVER
Research In Viral Eradication of HIV Reservoirs
A two-arm (proof of concept) randomised phase II trial

Protocol

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Date: 23-Jul-2015

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Signature:

Date:

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Role: Project Lead & Statistician

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Date

GENERAL INFORMATION

This document was constructed using the MRC CTU Protocol Template Version 3.0. The MRC CTU endorses the Standard Protocol Items: Recommendations For Interventional Trials (SPIRIT) initiative. It describes the **RIVER** trial, co-ordinated by the Medical Research Council (MRC) Clinical Trials Unit at UCL (herein referred to as MRC CTU), and provides information about procedures for entering participants into it. The protocol should not be used as an aide-memoire or guide for the treatment of other participants. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but sites entering participants for the first time are advised to contact RIVER Co-ordinating centre at, MRC CTU, to confirm they have the most up-to-date version.

COMPLIANCE

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki 1996, the principles of Good Clinical Practice (GCP), Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act (DPA number: Z6364106), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

SPONSOR

Imperial College London is the trial sponsor and has delegated responsibility for the overall management of the **RIVER** trial to the MRC CTU (RIVER Co-ordinating Centre). Queries relating to Imperial sponsorship of this trial should be addressed to the chief investigator via the Imperial Joint Research Compliance Office.

FUNDING

This study is funded by the Medical Research Council in collaboration with support from industry partners.

AUTHORISATIONS AND APPROVALS

The following persons are authorised to sign the final protocol and protocol amendments for the sponsor: Dr Sarah Fidler (Chief Investigator) and Professor Abdel Babiker (Trial Statistician).

TRIAL REGISTRATION

This trial has been registered with EUDRACT Clinical Trials Register, ISRCTN and clinicaltrials.gov.

TRIAL ADMINISTRATION

Please direct all queries to the Trial Manager at the RIVER Co-ordinating Centre in the first instance; clinical queries will be passed to the Trial Physician via the Trial Manager.

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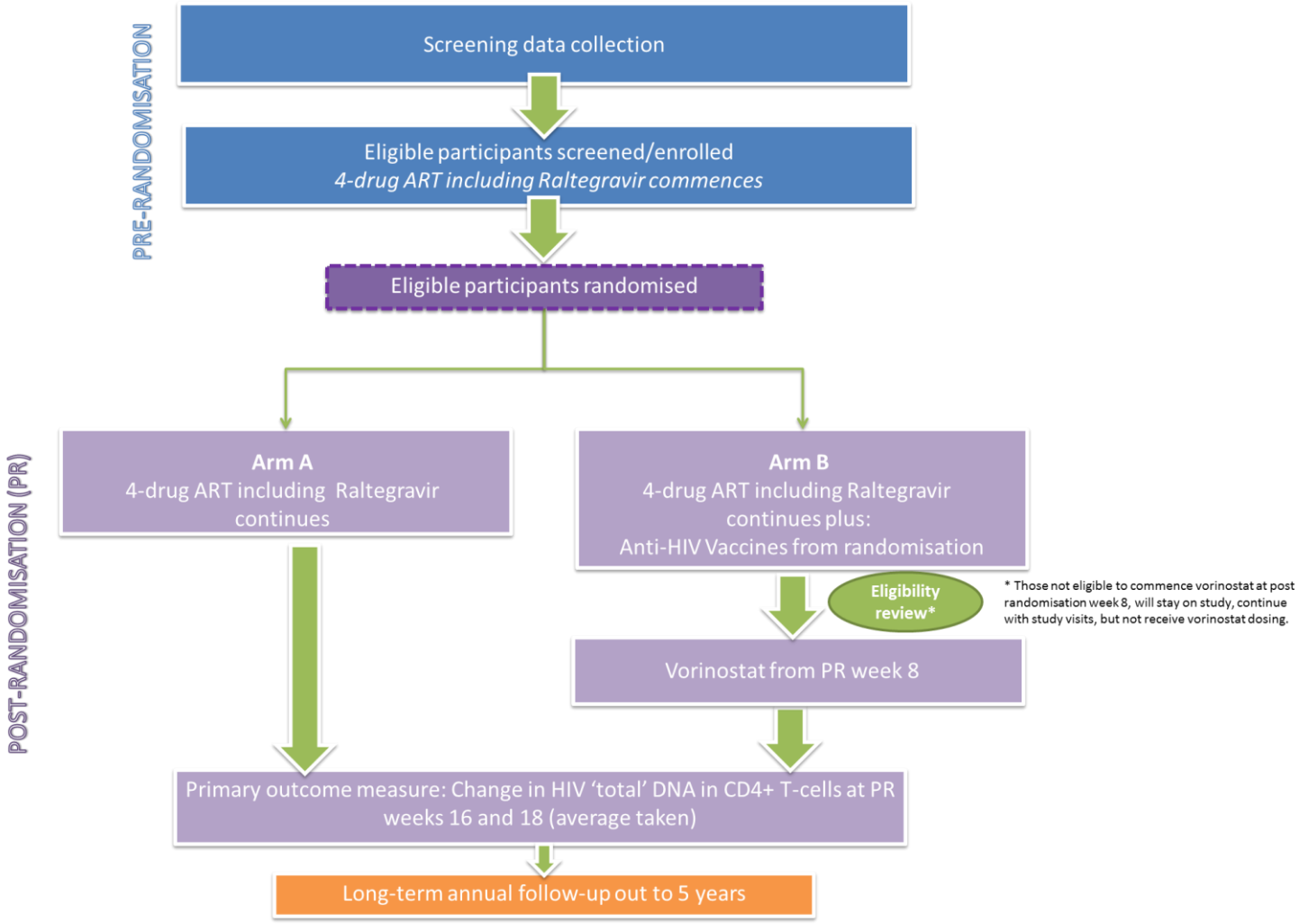
SUMMARY OF TRIAL

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
ACRONYM (or Short Title of Trial)	RIVER
Long Title of Trial	Research In Viral Eradication of HIV Reservoirs
Version	3.0
Date	23-Jul-2015
ISRCTN #	ISRCTN83717528
NCT #	NCT02336074
EudraCT #	2014-001425-32
Study Design	This study will be a two-arm prospective 1:1 phase II randomised controlled trial. Screening takes place during the 4 weeks following confirmation of primary HIV infection diagnosis. Eligible participants are enrolled at week 0 when combination ART (cART) treatment begins and randomisation of participants occurs after assessment of eligibility at week 22.
Participants	The study aims to randomise 52 eligible individuals across 6 UK collaborating centres according to the following criteria: 1. Aged ≥18 to ≤60 years old 2. Able to give informed written consent including consent to long-term follow-up 3. Should be enrolled within a maximum of 4 weeks of a diagnosis of primary HIV-1 infection confirmed by one of the following criteria: a. Positive HIV-1 serology within a maximum of 12 weeks of a documented negative HIV-1 serology test result (can include point of care test (POCT) using blood for both tests) b. A positive p24 antigen result and a negative HIV antibody test c. Negative antibody test with either detectable HIV RNA or proviral DNA d. PHE RITA test algorithm reported as "Incident" confirming the HIV-1 antibody avidity is consistent with recent infection (within the preceding 16 weeks). e. Weakly reactive or equivocal 4th generation HIV antibody antigen test f. Equivocal or reactive antibody test with <4 bands on western blot 4. Adequate haemoglobin (Hb≥12g/dL for males, ≥11g/dL for females) 5. Weight ≥50kg 6. Willing to start immediate cART and be randomised to continue cART alone or cART plus intervention (HIV vaccines plus HDACi) 7. Willing and able to comply with visit schedule and provide blood sampling Detailed inclusion/exclusion criteria can be found in **Section 3.**

SUMMARY INFORMATION TYPE	SUMMARY DETAILS
Interventions to be Compared	This study will be a two-arm prospective 1:1 randomised controlled trial comparing: Arm A: 4-drug cART including raltegravir (control) Arm B: 4-drug cART including raltegravir plus ChAdV63.HIVconsv (ChAd) prime and MVA.HIVconsv (MVA) boost vaccines; followed by a 28-day course of vorinostat (10 doses in total).
Study Hypothesis	In primary HIV infection, a combination of immediate cART, immunisation and latency reactivation using the HDACi vorinostat will confer a significant reduction in the reservoir when compared with cART alone.
Primary Outcome Measure(s)	The primary study outcome measure is HIV 'total' DNA from CD4 T-cells.
Secondary Outcome Measure(s)	Secondary outcome measure include: ▪ Clinical and laboratory adverse events,all grades, including SAE ▪ Further assessment of the HIV reservoir e.g. HIV integrated DNA; HIV cell associated RNA; plasma HIV RNA measured with an ultra-low copy assay i.e. with a threshold of <1 copy/ml, viral outgrowth assays ▪ Studies of immune function including measuring the latently-infected resting memory T-cells and cytotoxic immune responses ▪ Changes in inflammatory biomarkers
Randomisation	Participants will be randomised to either arm of the study in a 1:1 ratio once all inclusion criteria are met; in most cases this is anticipated to be about 23 weeks after enrolment.
Duration	Planned duration 2.5 years
Sponsor	Imperial College London
Funder	Medical Research Council & industry partners
Legal Representative in Europe	Imperial College London
Chief Investigator	Dr Sarah Fidler
MRC CTU Project Leader	Professor Abdel Babiker

TRIAL SCHEMA

Figure 1 Trial Entry, Randomisation and Treatment



TRIAL ASSESSMENT SCHEDULE & VISIT CODES

Table 1 Trial Schedule

Approx. Study Timeline (Weeks)		0	4	12	22	-	Rand	24	32							33							34							35							36	40	42
The length of the study period if randomisation takes place 24 weeks after enrolment.																																								
								POST-RANDOMISATION (PR)																														ENDPOINT	
STUDY VISITS	Screening (-4 to 0)	Enrolment Week 0	Week 4	Week 12	Week 22	Repeat Viral Load [b]	Randomisation [c]	PR Week 00 [d]	PR Week 08							PR Week 09							PR Week 10							PR Week 11							PR Week 12	PR Week 16	PR Week 18
DAY IN WEEK									1[f]	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2		
									Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Mon	Tue		
Visit Number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Where necessary	Visit 6	Visit 7	Visit 8		Visit 9						Visit 10						Visit 11							Visit 12								Visit 13	Visit 14	Visit 15
4-Drug ART Inc Raltegravir (Both Arms)		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Vaccine (Arm B only)								ChAd	MVA																															
								8 Weeks[e]																																
Vorinostat (Arm B only)											Dose 1			Dose 2			Dose 3			Dose 4			Dose 5			Dose 6			Dose 7			Dose 8			Dose 9			Dose 10		
											Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28		
Resting 12-lead ECG	✓				✓				✓																												✓		
EQ-5D-5L QOL Questionnaire		✓			✓		✓				✓																												✓
Hepatitis B & C serology (Results up to 2 months old can be used) [a]	✓																																						
Resistance test [a]	✓																																						
HCV PCR [a]	✓				✓																																		
Routine Study Bloods (34/27/25/14/7ml)	✓27		✓25	✓25	✓34	✓7	✓27		✓14		✓25						✓14						✓14							✓14							✓14	✓25	
Full Blood Count (haemoglobin, haematocrit, white blood cell count (WBC) with differential and platelets)	✓		✓	✓	✓		✓		✓		✓						✓						✓							✓							✓	✓	
Biochemistry (ALT, AST, alkaline phosphatase, total bilirubin and albumin; eGFR, creatine kinase)	✓		✓	✓	✓		✓		✓		✓						✓						✓							✓							✓	✓	
Biochemistry electrolytes i.e. Mg, Ca, K					✓		✓		✓		✓						✓						✓							✓							✓		
HbA1C (only Diabetics)	✓				✓		✓																																
Glucose	✓				✓		✓		✓		✓						✓						✓							✓							✓	✓	
Lipids (Total cholesterol, LDL, HDL, triglycerides)	✓				✓		✓																															✓	
Viral Load	✓		✓	✓	✓	✓	✓				✓																											✓	
T-Cell Counts	✓		✓	✓	✓		✓				✓																											✓	
Urinalysis (uPCR)	✓		✓	✓	✓		✓		✓		✓						✓						✓							✓							✓	✓	
Proviral DNA (total), Proviral DNA (integrated), Cell associated RNA , Genomics, Low Copy VL, HDAC Assay (60/80 ml) Oxford		✓80					✓80				✓60																										✓80	✓80	✓80
Replication competence and IUPM (250ml) Cambridge							✓250																															✓250	
Immunology (32/40ml) Oxford		✓32					✓32										✓32																				✓40	✓32	
Vorinostat related virology - Arm B only Cell associated RNA (20ml pre Vorinostat, 20ml 2hrs post (40ml) Oxford											✓40						✓40																				✓40		
Total Blood Draw (mL)	27	112	25	25	34	7	389	0	14		125						86						14							14							174	387	80

LONG-TERM ANNUAL FOLLOW-UP OUT TO 5 YEARS

Quarterly Blood Draw Totals (ml)	Quarter 1	Quarter 2	Quarter 3	Quarter 4	TOTAL
	189	430	427	467	1513

Schedule Notes:

a) Results for these tests should already be available. If they are not these samples need to be taken as part of screening.

b) If Viral Load is detectable at week 22 bloods, this may be repeated until the viral load falls below the level of detectability (<50copies/mL or <200copies/mL for the Taqman Roche 2.0 assay). All eligibility criteria must be assessed and met within 14 days prior to randomisation, and may need to be repeated.

c) The timing of the randomisation visit will be determined by the capacity of the vaccination centre

d) The first vaccination visit, PR00 , must take place within 1 week of randomisation and is for particpants randomised to Arm B only.

e) Time between the two vaccines is 8 weeks (+/- 7 days)

f) PR08-1 visit ideally takes place on a Monday but can be moved by up to 2 days, whilst still allowing all subsequent visits to fall on weekdays.

Table 2 – Visit names and codes

	Study visit name	Visit number	Visit schedule		Visit code
Pre – Randomisation	Screening	Visit 1	Eilgibility for enrolment assessed		Screening
	Enrolment	Visit 2	Participant is enrolled and commence 4-Drug ART including raltegravir (cART)		Week 0
	Follow up week 04	Visit 3	Follow up visit		Week 04
	Follow up week 12	Visit 4	Follow up visit		Week 12
	Follow up week 22	Visit 5	Eligibility for randomisation assessed		Week 22
	Randomisation	Visit 6	Participant is randomised		Randomisation
			Arm A (control) 4-Drug ART including raltegravir (cART)	**Arm B** 4-Drug ART including raltegravir (cART) + Vaccines + vorinostat	
Post – Randomisation	Post-randomisation week 00	Visit 7	Follow up visit	Administration of ChAd vaccine	PR00
	Post-randomisation week 08 Day 1	Visit 8	Follow up visit	Administration of MVA vaccine	PR08-1
	Post-randomisation week 08 Day 3	Visit 9	Follow up visit	First dose of vorinostat (then taken every 3rd day)	PR08-3
	Post-randomisation week 09 Day 2	Visit 10	Follow up visit	Vorinostat dosing continued	PR09-2
	Post-randomisation week 10 Day 1	Visit 11	Follow up visit	Vorinostat dosing continued	PR10-1
	Post-randomisation week 11 Day 1	Visit 12	Follow up visit	Vorinostat dosing continued	PR11-1
	Post-randomisation week 12 Day 2	Visit 13	Follow up visit	Last dose of vorinostat	PR12-2
	Post-randomisation week 16	Visit 14	Follow up visit	Follow up visit	PR16
	Post-randomisation week 18	Visit 15	Final follow up visit	Final follow up visit	PR18

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ABBREVIATIONS

ABBREVIATION	DEFINITION	ABBREVIATION	DEFINITION
ADI	AIDS-defining illness	GMFA	Gay Men Fight AIDS
AE	Adverse Event	GMO	Genetically modified organism
AHSC	Academic Health Science Centre	GMP	Good Manufacturing Practice
ANCOVA	Analysis of covariance	GSK	GlaxoSmithKline
AR	Adverse Reaction	HCV	Hepatitis C virus (HCV)
ART	Antiretroviral therapy	HDACi	Histone deacetylase inhibitors
AUC	Area under the curve	HIV-1	Human Immunodeficiency Virus Type 1 (HIV-1)
BHIVA	British HIV Association	HTLV-1	Human T Cell Lymphotropic Virus-1
BID	Twice a day	IB	Investigator Brochure
CAB	Community Advisory Board	IDMC	Independent Data Monitoring Committee
cART	Combination Antiretroviral Therapy	IL-7	Interleukin 7
CBF	Clinical BioManufacturing Facility	IM	Intra-muscularly
CHERUB	Collaborative HIV Eradication of viral Reservoirs	INSTI	Integrase Strand Transfer Inhibitors
CHO	Chinese Hamster Ovary	ITT	Intention to Treat
CNS	Central Nervous System	IVIG	Intravenous immunoglobulin
CRF	Case Report Form	LFT	Liver Function Tests
CTA	Clinical Trials Authorisation	LPLV	Last participant last visit
CTCL	Cutaneous T cell lymphoma	MHRA	Medicines and Healthcare Products Regulatory Agency
CTU	Clinical Trials Unit	MSD	Merck Sharp and Dohme
CYP	Cytochromes P450	MRC	Medical Research Council
DDI	Drug-to-drug interaction	MRC CTU	Medical Research Council Clinical Trials Unit at UCL
DNA	Deoxyribonucleic acid	NGO	Non-governmental organisation
DPA	Data Protection Act	NHS	National Health Service
DSUR	Development Safety Update Report	NNRTI	Non-nucleoside reverse transcriptase inhibitors
EBV	Estimation of blood volume	NRTI	Nucleoside/nucleotide reverse transcriptase inhibitors
ECG	Electrocardiography	NSAID	Non-Steroidal Anti-Inflammatory Drug
eGFR	Estimated Glomerular Filtration Rate	OD	Once a day
EU	European Union	PBMC	Peripheral blood mononucleated cell
FBC	Full Blood Count	PHE	Public Health England
GCP	Good Clinical Practice	PHI	Primary HIV-1 infection

ABBREVIATION	DEFINITION	ABBREVIATION	DEFINITION
PI	Protease Inhibitor	SAE	Serious Adverse Event
PIL	Patient Information Leaflet	SAP	Statistical Analysis Plan
PIM	Protocol Instruction Manual	SAR	Serious Adverse Reaction
PI/r	Ritonavir-boosted PI	SIV	Simian immunodeficiency virus
PK	Pharmacokinetics	SmPC	Summary of product characteristics
POCT	Point-of-care testing	SNA	Serious Non-AIDS Event
PR	Post-Randomisation	SUSAR	Suspected Unexpected Serious Adverse Reaction
QA/QC	Quality Assurance/Quality Control	THT	Terrance Higgins Trust
QD	Quaque die (one a day)	TMF	Trial Master File
R&D	Research and Development	TSC	Trial Steering Committee
REC	Research Ethics Committee	UAR	Unexpected Adverse Reaction
RGF	Research Governance Framework	UK BRC	UK Biomedical Research Centres
RITA	Recent Infection Testing Algorithm	UK DPA	UK Data Protection Act
RIVER	Research In Viral Eradication of HIV Reservoirs	uPCR	Urine protein to creatinine ratio
RNA	Ribonucleic acid	ULN	Upper Limit of Normal
SAHA	Suberoylanilide hydroxamic acid	VL	HIV viral load

1 BACKGROUND

Although antiretroviral therapy (ART) has significantly improved long-term survival for people living with HIV by controlling viral replication, it alone cannot 'cure' infection. This is because ART alone is not capable of eradicating virus from all infected cells, and so in chronic stages of HIV disease, for the majority of individuals cessation of ART leads to immediate recrudescence of viraemia[1].

There is broad consensus that following HIV transmission (acute infection or HIV seroconversion), the first viraemia of HIV infection leads to the seeding of the HIV genome as integrated proviral DNA in all CD4-bearing permissive cells, predominantly resting memory CD4+ T-cells and other long-lived cells, such as macrophages[2-4]. After the initial viraemia has settled, many of these provirally-infected cells will revert to a resting state where they become non-activated and non-replicating and where the viral genome is transcriptionally silent (viral latency). These infected cells are termed the HIV reservoir or latently-infected pool, and it is the failure to eradicate these cells that means ART is unable to cure infection. As these latently-infected cells do not express any viral antigens they cannot be recognised or eliminated by T cell surveillance and ART does not affect them. As many latently-infected cells may be long-lived, there is lifelong persistence of HIV in the face of suppressive ART.

Latently infected cells will randomly become activated over time and viral particles may be produced, although with continued ART viral progeny will not be able to propagate. However, the observation that viral recrudescence is almost universal on cessation of ART[1] suggests that the half-life of these latently-infected cells must be very long.

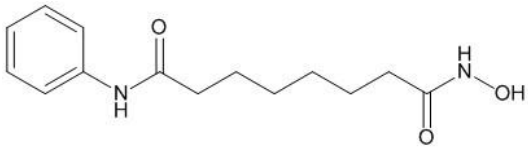
Maintenance of viral latency is a complex process where several factors restrict completion of viral replication. Latency is maintained in part by the activity of histone deacetylase (HDAC)[5], an enzyme that removes acetyl groups from DNA-bound histone proteins and, in so doing, affects gene expression.

The only way to 'cure' HIV infection, defined as the absence of any detectable infected cells off ART, has been to use a complex intervention including bone marrow transplantation, total body irradiation and systemic chemotherapy[6] which is not feasible or scaleable but provides a fascinating proof of concept. The much cited 'Mississippi baby' case gave some hope that starting immediate ART within hours of estimated vertical transmission might prevent the establishment of HIV infection. Unfortunately, HIV has now rebounded in this child, and subsequently one other similar case. indicating that despite very early antiretroviral therapy and no initial rebound of virus off treatment, that this child is truly HIV infected[7]. Alternative strategies more amenable to generalizable use are the strategic use of ART as near to the defined time of viral acquisition[8]. In one cohort of patients (VISCONTI) treated with ART during primary infection, 15.6% had no rebound of virus following treatment interruption, however, in the majority, virus rebounded and became detectable on stopping ART. It appears therefore that for the majority of individuals a further intervention in addition to ART maybe required to confer viral control off therapy, even for those starting as close to the time of viral transmission as possible. Elimination of the HIV reservoir is likely to require more than one intervention: 1) suppression of viral replication through ART; 2) re-activation of HIV transcription from latently-infected cells and 3) elimination of these reactivated cells; the 'shock and kill' strategy[9, 10].

Re-activation of latently-infected cells results in viral transcription and hence is likely to lead to expression of viral antigens. One approach currently being explored to eliminate latently-infected CD4+ T-cells is to activate viral production from these cells in the presence of ART. The rationale for this approach is that production of virus should kill the infected cells and subsequent rounds of infection would be inhibited by the presence of ART. Based upon this principle, initial proof of concept studies have explored the role of adding novel agents capable of inducing viral transcription to ART; including anti-PD-1, IL-7, intravenous immunoglobulin (IVIG), gene therapy, prostratin and histone deacetylase inhibitors (HDACi)[4, 10-12].

1.1 RESEARCH TREATMENT: HISTONE DEACETYLASE INHIBITORS (HDACI)

HDACi are a new class of anti-neoplastic agents, which have undergone rapid development in several phase I/II studies. Vorinostat (suberoylanilide hydroxamic acid abbreviated to SAHA) inhibits the histone deacetylases HDAC1, HDAC2, HDAC3 (Class I) and HDAC6 (Class II).



Vorinostat (Zolinza™) is supplied as capsules containing 100mg vorinostat and the following inactive ingredients: microcrystalline cellulose, sodium croscarmellose and magnesium stearate. The capsule shell excipients are titanium dioxide, gelatin and sodium lauryl sulphate.

Figure 2 Vorinostat Structure

1.1.1 PRE-CLINICAL SAFETY DATA

Vorinostat was mutagenic in vitro in the bacterial reverse mutation assays (Ames test), caused chromosomal aberrations in vitro in Chinese hamster ovary (CHO) cells and increased the incidence of micro-nucleated erythrocytes when administered to mice (mouse micronucleus assay). Effects on the female reproductive system were identified in the oral fertility study when females were dosed for 14 days prior to mating through gestational day 7. Doses of 15, 50 and 150mg/kg/day to rats resulted in approximate exposures of 0.15, 0.36 and 0.70 times the expected clinical exposure based on AUC. Dose dependent increases in corpora lutea were noted at ≥15mg/kg/day and increased peri-implantation losses were noted at ≥50mg/kg/day. At 150mg/kg/day, there were increases in the incidences of dead foetuses and in resorptions. No effects on reproductive performance observed in male rats dosed (20, 50, 150mg/kg/day; approximate exposures of 0.15, 0.36 and 0.70 times the expected clinical exposure based on AUC), for 70 days prior to mating with untreated females.

1.1.1.A PHARMACOKINETICS (PK)

Vorinostat is absorbed orally and absorption is increased (33%) by co-absorption of dietary fat. Administration of vorinostat with food achieves maximum concentration at a median of 4 (0.5-14) hours. Vorinostat is ≈71% bound to human plasma proteins; mean terminal half-life (t½) is ~2.0 hours. Elimination is predominantly through metabolism to two inactive metabolites by glucuronidation and hydrolysis followed by ß-oxidation. While vorinostat has not been evaluated in patients with hepatic or renal insufficiency, renal excretion does not play a role in the elimination of vorinostat. Based upon an exploratory analysis of limited data, gender, race and age do not appear to have meaningful effects on the PK; vorinostat has not been evaluated in patients <18 years of age. Vorinostat is not eliminated via the CYP pathways, neither is it an inhibitor of CYP drug metabolising enzymes in human liver microsomes at steady state. Some suppression of CYP2C9 and CYP3A4 activity was detected at concentrations higher (≥10µM) than pharmacologically relevant. It is not anticipated that vorinostat will be subject to drug-drug interactions (DDI) when co-administered with drugs that are known CYP inhibitors or inducers. However, no formal DDI clinical studies have

been conducted to evaluate drug interactions with vorinostat. In vitro studies indicate that vorinostat is not a substrate of human P-glycoprotein (P-gp). In addition, vorinostat has no inhibitory effect on human P-gp mediated transport of vinblastine (a marker P-gp substrate) at concentrations of ≤100µM. Thus, vorinostat is not likely to inhibit P-gp at pharmacologically relevant serum concentrations of 2µM (C_{max}) in humans. There does, however, appear to be an interaction with warfarin (coumarin) with prolongation of INR (international normalized ratio).

1.1.1.B PHARMACOKINETIC/PHARMACODYNAMIC – EFFECTS ON QTC

In 25 healthy volunteers given a single supratherapeutic dose of vorinostat 800mg, no patient had a significant change in QTc over 24 hours of ECG monitoring. In other studies three of 86 cutaneous T cell lymphoma (CTCL) patients exposed to 400mg QD had Grade 1-2 QTc prolongation. In a retrospective analysis of 5 studies, 4 of 116 evaluable patients had grade 2 QTc prolongation, and 1/116 had grade 3 QTc prolongation. Of 49 non-CTCL patients from 3 clinical trials 1 had grade 2 prolongation and 2 had grade 3 QTc prolongation.

1.1.1.C CLINICAL EXPERIENCE WITH HDACI IN OTHER DISEASE AREAS

HDACi have shown efficacy in relapsed/refractory myelodysplasia (MDS), acute myeloid leukaemia Hodgkin lymphoma (HL) and in particular the T cell lymphomas[13]. In the US vorinostat is licensed (since 2006) for the treatment of cutaneous manifestation of CTCL in those who have progressive, persistent or recurrent disease on or following two systemic therapies. It has also been granted orphan drug status for the treatment of refractory multiple myeloma in the US (October 2013). In the EMEA, vorinostat was granted orphan designation for use in malignant mesothelioma in September 2010; but this was withdrawn at the Sponsor (MSD) in February 2013. Other HDACi such as romidepsin (depsipeptide), panobinostat and MGCD-0103 have also demonstrated activity in various haematological malignancies. Further information on the toxicity profile of vorinostat is provided in **Appendix II** but in summary – in the cancer setting, the most common drug-related adverse reactions can be classified into four symptom complexes.

1. Gastrointestinal symptoms - Anorexia, nausea, vomiting, diarrhoea, weight loss and constipation.
2. Constitutional symptoms - Fatigue has been reported commonly with use of vorinostat and occurred more commonly at doses of vorinostat >400mg per day. Chills and fever have also been reported.
3. Abnormalities - Dose-related anaemia and thrombocytopaenia can occur with vorinostat and are more common at doses of >400mg per day. Neutropaenia is uncommon, but has been reported. Monitoring of blood cell counts should be performed every 2 weeks during the first 2 months of therapy and monthly thereafter. If platelet counts and/or haemoglobin are reduced during treatment with vorinostat, the dose should be modified or therapy discontinued.
4. Taste disorders - Dysgeusia and dry mouth have been reported.

The most common serious adverse events (SAE), regardless of causality, in the 86 CTCL patients in two clinical studies were pulmonary embolism reported in 4.7% (4/86) of patients, squamous cell carcinoma reported in 3.5% (3/86) of patients and anaemia reported in 2.3% (2/86) of patients. Patients on vorinostat should therefore be monitored for pertinent signs or symptoms of deep vein thrombosis or pulmonary embolism.

1.1.1.D DISCONTINUATION DUE TO ADVERSE EVENTS IN CTCL PATIENTS WHO RECEIVED THE 400MG QD DOSE

9.3% (8/86) discontinued vorinostat due to AE and 10.5% (9/86) required a dose modification due to AE. The median time to the first AE resulting in dose reduction was 42 days (range 17 to 263 days).

Laboratory abnormalities were reported in the 86 patients who received the 400-mg dose and one patient who received a 350-mg dose. Increased serum glucose was detected by laboratory safety tests in 69% (60/86) of CTCL patients, but was severe (Grade 3) in only 5 of these. Hyperglycaemia was reported as a drug-related adverse event in in 4.7% (4/86of CTCL patients receiving 400mg QD. Transient increases in serum creatinine were detected in 47.1% (41/87) of CTCL patients; in most cases these increases were non-severe, however, Grade 3 AEs have been observed. Based on reports of dehydration as a serious drug-related adverse experience in clinical trials, patients were instructed to drink at least 2 L/day of fluids for adequate hydration. After these precautions were implemented, the incidence of dehydration decreased. Proteinuria was detected as a laboratory abnormality (51.4%) in 38 of 74 patients tested. The clinical significance of this finding is unknown.

1.1.2 Rationale and Clinical Experience with the HDACi in HIV-infection

Although some (HDACi) can induce mutations (in vitro), they may reverse latency[5, 11]. Initial studies using HDACis such as sodium valproate with ART in HIV failed to limit the reservoir size, or confer clinical advantage[14]. Vorinostat better targets the specific histones associated with HIV repression and reactivates HIV from latently-infected T cell lines and primary T-cells infected in vitro and in vivo[15, 16]. Transient hyperacetylation of histones in peripheral blood mononuclear cells (PBMC) and tumour cells occurred two hours after in vivo administration and returned to baseline levels eight hours post treatment[17] . A single dose proof-of-concept trial in chronically HIV infected individuals, showed that vorinostat with ART resulted in a 5-fold increase in viral transcription from the reservoir[18]. More recently, a 14 day dosing schedule of vorinostat in chronically HIV infected individuals on ART induced a 2.5-fold increase in viral transcription[19]. Recent data from Margolis *et al*[20] has shown that for some individuals there is a refractory period after dosing with vorinostat during which there is no measureable increase in histone deacetylation inhibition. In addition, in vitro cell culture models have similarly demonstrated a potential refractory period after vorinostat dosing of between 24-28 hours. Whilst daily oral dosing was used in the Lewin 14 day course with some but limited effect on histone deacetylation inhibition, we have altered our dosing schedule to every 3 days to try and accommodate this potential refractory period. Furthermore, we anticipate that reduced dosing frequency may limit toxicity and adverse events. These data provide proof-of-concept for HDACi as a potential therapeutic class in HIV to activate latent HIV infection in humans. Vorinostat is currently not licensed for the treatment for HIV.

From the two clinical studies employing vorinostat with ART, there is evidence that vorinostat stimulated viral transcription from latently-infected cells. However, there was no evidence of alteration in replication competent viral expression or total proviral DNA, suggesting that reactivation of viral transcription was not accompanied by cell death[9]. An in vitro model of viral latency using infected resting CD4+ T-cells from HIV positive individuals on ART showed that reversal of latency using vorinostat failed to lead to cell death, even in the presence of autologous cytolytic T lymphocytes (CTLs) isolated from patients on ART. However, addition of autologous antigen-specific stimulation of HIV positive patients' CTLs to the in vitro latency model after activation with HDACi led to efficient killing of infected cells. These results demonstrate that stimulating HIV-1-specific CTLs prior to reactivating latent HIV-1 may be essential for successful eradication efforts[21]. It is for this reason that we propose a unique immunisation strategy as an adjunct to HDACi administration in vivo.

More information on the tolerability of 14 days of daily dosing in chronically infected HIV-infected patients on combination antiretroviral therapy (cART) can be found in **Appendix II.**

1.2 RESEARCH TREATMENT: HIV VACCINES

1.2.1 THE DEVELOPMENT OF UNIQUE HIV-1 IMMUNOGENS

The selection of viral sequences for inclusion in an HIV-1 immunogen in the setting of a therapeutic "viral activation" is challenging since an immune correlate of viral reservoir expression as well as protection is lacking and the immunodominant responses detected in HIV+ patients in chronic infection may have little impact on virus control in ART treated seroconverters. The extreme variability of HIV-1 enables rapid escape from cell-mediated and humoral immunity, and is thus a major obstacle to the development of an effective vaccine. To overcome this challenge, a unique 'universal' T cell immunogen (HIVconsv) has been developed at the University of Oxford that is comprised of the 14 most highly conserved regions of the HIV-1 proteome assembled into a single chimaeric protein[22]. HIVconsv is encoded by a 2.4kbp gene as a fusion protein and contains approximately 25% of the known HIV-1 CD8+ T cell epitopes, of which 70% are identical between the major clades. Each segment is a consensus sequence from one of the four major HIV-1 clades, A, B, C and D, which alternate to ensure equal clade coverage. Epitopes recognised by rhesus macaque and mouse CD8+ T-cells were added to the C-terminus to facilitate pre-clinical development. The gene coding for HIVconsv was inserted into the three most studied vaccine vectors, plasmid DNA, human Ad5 and MVA, and tested for immunogenicity in Balb/c and HLA-A2 transgenic (HHD) mice. A regimen comprising the DNA, Ad5, MVA (DAM) sequence of vaccinations induced CD8+ T cell responses >2000 spot-forming units (SFU)/million PBMCs in IFN-ɣ ELISpot assays and was superior to other combinations of these vaccines[22]. Peptides based on HIVconsv stimulated strong and broad responses in IFN-ɣ ELISpot assays with PBMC from HIV-1-infected individuals, indicating that human T-cells which have been primed by HIV-1 can recognise HIVconsv-derived epitopes.

1.2.2 DEVELOPMENT OF HIGHLY IMMUNOGENIC REPLICATION-DEFECTIVE SIMIAN ADENOVIRAL VECTORS

Replication-defective adenoviruses are currently the most potent vectors for induction of human T cell responses to encoded antigens. They are easily propagated in cell culture to high titres, are relatively inexpensive to manufacture and have an excellent safety profile[23]. However, the use of human Ad vectors is limited by pre-existing humoral anti-vector immunity, with 60% and 90% anti-Ad5 seroprevalence rates in Europe and sub-Saharan Africa, respectively[24]. The prevalence of immunity to human Ad has prompted the consideration of simian adenoviruses as vectors as these have low or no seroprevalence in the human population (less than 5% in humans residing in the USA[25]. In addition, Simian adenoviruses are not known to cause disease in humans. To identify novel vaccine carriers suitable for vaccine delivery in humans, Okairos Srl isolated and sequenced over a thousand Adenovirus strains from chimpanzees (ChAd or AdCh)[26]. Replication-defective vectors were generated and screened for neutralisation by human sera and for ability to grow in human cell lines that were approved for clinical studies. The vectors studied varied up to a thousand-fold in potency for CD8+ T cell induction in mice. The most potent ChAd vectors were selected for Phase I clinical studies at the University of Oxford, where it was shown that ChAd-vectored vaccines for malaria, HCV and HIV-1 infections are safe and highly immunogenic[27-29]

1.2.3 MODIFIED VACCINIA VIRUS ANKARA (MVA) AS A VIRAL VECTOR

Modified vaccinia virus Ankara (MVA) is a vaccinia virus strain which was attenuated by serial passage in chick embryo fibroblasts (CEF). It has lost 15% of the parental genome, including cytokine receptor genes. It replicates well in CEF and baby hamster cells but poorly in most mammalian cells[30, 31]. It was used as a smallpox vaccine in the early 1970s, towards the end of the eradication campaign, in 120,000 people without any serious adverse event. Its safety in humans is therefore well established.

MVA has been shown to be effective as a vaccine vector; in particular, recombinant MVA is capable of inducing potent CD8+ T cell responses to passenger proteins, particularly when used as a booster in prime-boost regimens. The immunogenicity of MVA has been attributed in part to loss of cytokine and chemokine receptor genes[32]. MVA-vectored vaccines expressing SIV (simian immunodeficiency virus) genes have been evaluated in the macaque model, either as a single vaccine modality or in prime-boost regiments. Vaccination followed by a pathogenic SIV challenge rarely gave complete protection against infection but strong virus-specific CD8+ T cell responses were observed[33]. In some monkeys, containment of infection, indicated by significantly lower virus set points compared with sham-vaccinated animals, were observed after infection with the challenge virus[34, 35].

1.2.4 PRE-CLINICAL DATA

The Investigator Brochures for the vaccines detail the pre-clinical and clinical experience to date with the vaccine proposed for use in RIVER. Please refer to the respective Investigator's Brochure for detailed information.

Available pre-clinical data for each vaccine candidate is summarised below.

1.2.4.A CHADV63.HIVCONSV

In pre-clinical studies in mice and rhesus macaques, the ChAdV63.HIVconsv vaccine was found to be safe and immunogenic. In a dose escalation experiment in mice (10^5 to 10^9 virus particles per dose), the frequencies of HIV-1-specific T-cells increased in a dose-dependent manner. In macaques, the ChAdV63.HIVconsv vaccine significantly boosted transgene-specific T-cells in optimally-primed animals.

A pre-clinical GLP toxicology study carried out by Huntingdon Life Sciences, study UNO0012, assessed in groups of 10 male and 10 female BALB/c mice the systemic toxic potential following administration of 50µg dose of the DNA vaccine pSG2.HIVconsv ('D') by intramuscular injection on days 1, 15 and 29, followed by an intramuscular injection of $5x10^9$ viral particles (vp) ChAdV63.HIVconsv ('C') on day 43 (the 'DDDC' regimen). Vaccinations were not found to be associated with any systemic toxicological changes and all the observed lymph node changes were consistent with response to vaccine administration.

1.2.4.B MVA.HIVCONSV

Study UNO011 was performed by Huntingdon Life Sciences to assess the systemic toxic potential of 2×10^7 pfu/dose of MVA.HIVconsv, administered by intramuscular injection to groups of 10 male and 10 female BALB/c mice on days 1, 15 and 29 (the mMM regimen). Vaccinations were not found to be associated with any systemic toxicological changes. The findings of increased cellularity of the draining lymph nodes, high plasma gamma globulin, concentration and aspartate aminotransferase activity and inflammatory changes at the dose sites were considered to be consistent with a predicted response to vaccine administration. These results supported the MHRA approval for clinical trial HIV-CORE 001.

1.2.5 CLINICAL DATA

1.2.5.A CHADV63.HIVCONSV

In trial HIV-CORE 002, conducted at the University of Oxford, the safety and immunogenicity of three HIVconsv vaccine regimens, with and without DNA priming, was investigated in healthy volunteers. The three vaccines administered during the study were as follows: pSG2.HIVconsv DNA (D), ChAdV63.HIVconsv (C) and MVA.HIVconsv (M). Two volunteers received a single dose of ChAdV63.HIVconsv ($5x10^9$ vp) in an initial safety evaluation of the ChAdV63 vector. Thirty volunteers

were then recruited and assigned sequentially in groups of 10 to one of three vaccination regimens: CM (weeks 0 and 8), DDDCM (weeks 0, 4, 8, 12 and 20) or DDDMC (weeks 0, 4, 8, 12 and 16). Within each group, subjects were randomised to receive vaccine or placebo in a 4:1 ratio. Vaccine doses were: ChAdV63.HIVconsv $5x10^{10}$ vp, MVA.HIVconsv $2x10^{8}$ pfu, pSG2.HIVconsv DNA 4mg. All vaccines were given via the intramuscular route and each dose divided between two arms. 28/30 subjects completed the vaccination schedule; two subjects withdrew after receiving one vaccination, for reasons unrelated to safety or tolerability.

Overall, safety and tolerability profiles were excellent[36]. There were no suspected unexpected serious adverse reactions (SUSARs) and the majority of local and systemic vaccination reactions were grade 1 and lasted less than 48 hours (erythema/pain at the injection site, myalgia, 'flu-like' symptoms, headache). One volunteer experienced fever >38.5°C and there was one SAE that was unrelated to any of the investigational vaccines (appendicitis occurring after enrolment and before receipt of any vaccine). Additionally, a further trial has received approval to test pSG2.HIVconsv DNA (D) and ChAdV63.HIVconsv (C) in the format DDDC (HIV-CORE 003). This is a randomised double-blind placebo-controlled phase I/IIa trial to investigate the effect of depletion of serum amyloid P component (SAP) on the immune response to DNA vaccination in healthy male volunteers.

1.2.5.B MVA.HIVCONSV

MVA.HIVconsv has been evaluated in two phase I studies conducted at the University of Oxford. HIV-CORE 001 tested the safety and immunogenicity of three MVA.HIVconsv immunisations, administered by intramuscular injection to 19 HIV-1 seropositive patients on highly active anti-retroviral therapy (HAART)[37]. Ten volunteers were randomised to receive either MVA.HIVconsv ($5x10^{7}$ pfu) or placebo in a 4:1 ratio. A further 9 volunteers were randomised to receive either MVA.HIVconsv ($2x10^{8}$ pfu) or placebo in a 4:1 ratio. All 19 patients received at least two doses of MVA.HIVconsv and 17 patients completed their allocated vaccine regimen. The vaccine was well tolerated and there have been no SARs or SUSARs to date. There have been four SAE in three volunteers, none considered related to the vaccine (pneumonia, pulmonary abscess, pyelonephritis and Hodgkin's lymphoma). A blinded analysis of the data indicates that CD8+ T cell responses to highly conserved HIV-1 epitopes, which were primed by natural infection, were both boosted and broadened, with epitopes across the entire immunogen being targeted.

1.2.5.C USE OF CHADV63.HIVCONSV AND MVA.HIVCONSV IN HIV INFECTION

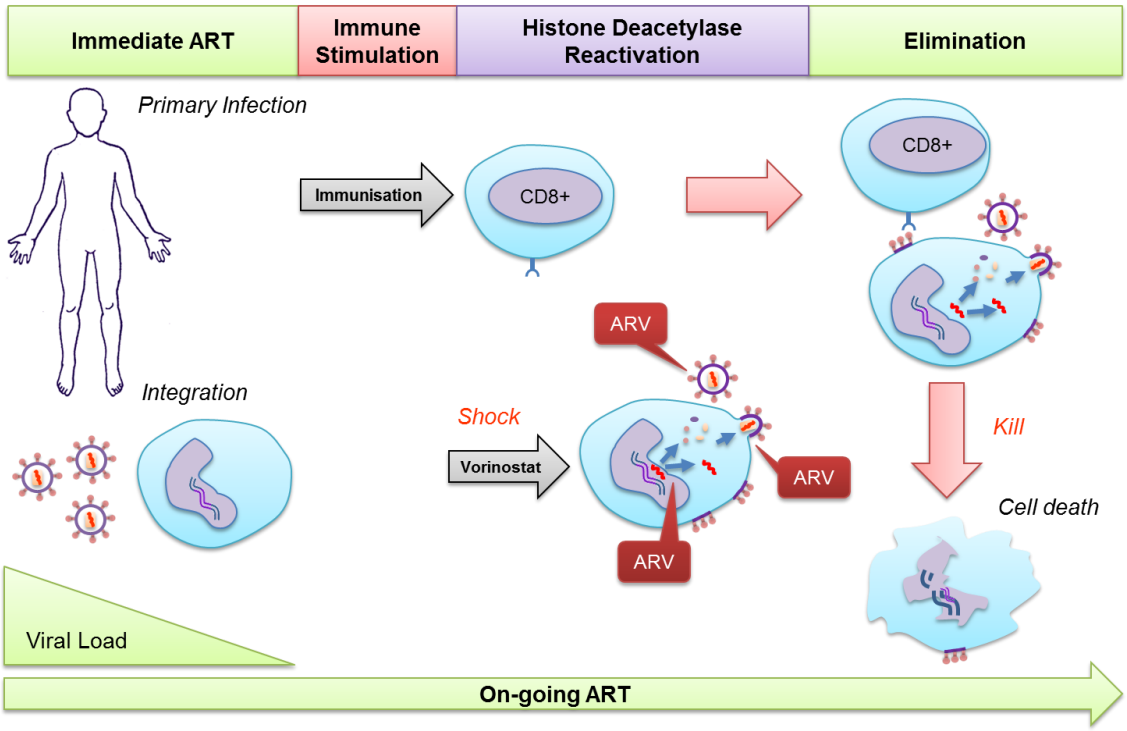
In the trial BCN01, which was conducted at the Irsicaixa AIDS Research Institute-HIVACAT, Barcelona, the safety and immunogenicity of ChAdV63.HIVconsv prime followed by MVA.HIVconsv boost vaccinations was assessed in early ART-treated HIV-1-positive individuals (NCT01712425)[38]. 24 individuals with recent HIV infection were treated immediately (median 91 days since HIV acquisition) with Tenofovir / Emtricitabine / Raltegravir for 6 months and then allocated to receive ChAdV63.HIVconsv (5x1010vp) followed by MVA.HIVconsv (2x108pfu) after either a 24-week (n = 12) or 8-week (n = 12) interval. Both vacccinations were given by intramuscular injection. Participants remained on ART throughout follow-up. Local and systemic events were recorded for a minimum of 7 days following each immunisation and were observed in 22/24 subjects. The majority were grade 1 or 2. One individual experienced grade 3 pain after each immunisation; grade 3 systemic symptoms were observed in one subject after ChADV63.HIVconsv and 3 subjects after MVA.HIVconsv. Pain was more frequent after MVA.HIVconsv than after ChAdV63.HIVconsv, which was consistent with previous trials. There have been no SUSARs or vaccine-related SARs. Analysis of immunogenicity and of viral reservoir parameters is ongoing (and will be presented at IAS 2015) [50].

1.3 STUDY DESIGN AND HYPOTHESIS

The study design is a two-arm, open label randomised study. Eligible participants will be treatment-naïve HIV-1 infected adults with primary infection. All participants receive 4-drug combination ART (cART) for the duration of the intervention phase of the study (42 weeks). In patients meeting the criteria for randomisation (eligibility assessed at week 22), participants will either continue cART or receive an intervention consisting of two anti-HIV vaccines separated by 8 weeks followed by 10 doses of the HDACi, vorinostat, in addition to cART. We hypothesise that the prime-boost vaccination will result in the generation of vaccine induced HIV specific CTLs that will recognise HDACi-activated cells of the HIV reservoir and destroy them. The net effect will be a greater reduction in the HIV reservoir defined as HIV total DNA in CD4+ T-cells in the cART+vaccine+HDACi compared to the cART alone. Our strategy is entirely different from previous therapeutic vaccination approaches which have been largely unsuccessful[39,40]. Immunological priming to conserved HIV proteins will drive CD8+ T-lymphocyte recognition of latently-infected cells rendered immunogenic by HDACi. We anticipate that the viral antigens expressed by latently-infected cells will be unable to adapt to, or escape from, the immune response as they will be expressed directly from chromosomal DNA, avoiding the steps of the viral life-cycle that facilitate immune-driven adaptation. We have chosen a prime-boost immunisation strategy with recombinant replication-defective chimpanzee adenovirus and modified vaccinia Ankara vectors, bearing conserved HIV antigens; these products have been shown to induce high titres of HIV-specific CD8+ T-cells[29, 41]. In addition, these vaccines will drive immune responses against conserved regions of the virus that may be well preserved in individuals with PHI.

Primary HIV Infection (PHI) is a unique period when HIV proviral reservoir is smaller than in chronic disease, is likely to be more homogeneous than in later stage disease and hence is more susceptible to immunological elimination. This provides an opportunity to use a vaccine to re-direct HIV-specific immune responses towards genetically fragile regions in the viral proteome. Immunisation in PHI should result in potent immune responses because ART initiated in PHI preserves CD4 function[42] and early ART-mediated viral suppression limits viral diversification, reducing the chance of immune escape[43]. The other key reason for conducting this trial in PHI is that, in some patients, an early sustained course of ART started very early in infection may induce a state of viral remission in which therapy can be stopped without any rebound viraemia. This has been most notably reported in the VISCONTI cohort[44] in which 'post-treatment control' was identified in 15.6% of selected individuals.

Figure 3 Pathway to an HIV cure. The triple combination of cART, immunisation and latency reversal should lead to T cell-induced killing of the HIV reservoir.



We hypothesise that the combination of HDACi with immunisation in cART-suppressed PHI will significantly impact the HIV reservoir.

Figure 3 illustrates the rationale for the interventions in the study.

1) All patients enrolled will receive combination antiretroviral therapy designed to reduce the plasma viral load as quickly as possible, hence the inclusion of the integrase inhibitor, raltegravir. Treating in PHI may restrict the size of the reservoir.
2) The prime-boost vaccination is designed to enhance the killing capacity of the cytotoxic T-cells. This must be given before the HDACi in order to prime and boost a maximal HIV-specific T-cell response to recognise activated viral antigen expression on reservoir cells.
3) The HDACi is designed to activate the reservoir, and in the presence of the enhanced killing capacity of the CD8+ T-cells, results in killing of the cells previously harbouring latent virus, leading to further reductions in the reservoir.

This combined approach has never been used, and we hypothesise there will be a 50% reduction in the proviral DNA (the 'reservoir'), in this 'proof-of-concept' study, in those randomised to the vaccine-HDACi intervention compared to those receiving antiretroviral therapy alone.

2 SELECTION OF SITES/CLINICIANS

The trial sponsor (Imperial College London) has overall responsibility for site and investigator selection.

2.1 SITE/INVESTIGATOR INCLUSION CRITERIA

To participate in the RIVER trial, investigators and clinical trial sites have fulfilled a set of basic criteria that have been agreed by the RIVER Trial Management Group (TMG) and are defined below.

The clinical sites included in this protocol have previously been part of a successful collaborative effort to enrol individuals presenting with acute HIV infection[45]. The CHERUB collaboration (Collaborative HIV Eradication of viral Reservoirs: UK BRC) is a unique collective effort between the 5 key Academic Health Science Centres (AHSC) in the UK who have been working together for the past 3 years. to develop laboratory and clinical expertise in the field of HIV cure research[46].

2.1.1 PRINCIPAL INVESTIGATOR'S QUALIFICATIONS & AGREEMENTS

1. The investigator should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial at their site and should provide evidence of such qualifications through an up-to-date curriculum vitae and/or other relevant documentation requested by the Sponsor, the REC, and/or the regulatory authority.
2. The investigator should be thoroughly familiar with the appropriate use of the investigational product, as described in the protocol, in the current Investigator Brochure, in the product information and in other information sources provided by the Sponsor.
3. The investigator should be aware of, and should comply with, the principles of GCP and the applicable regulatory requirements. A record of GCP training should be accessible for all investigators.
4. The investigator/site should permit monitoring and auditing by the Sponsor, and inspection by the appropriate regulatory authority
5. The investigator should maintain a delegation log of appropriately-qualified persons to whom the investigator has delegated significant trial-related duties.
6. The investigator should sign an investigator statement, which verifies that the site is willing and able to comply with the requirements of the trial.

2.1.2 ADEQUATE RESOURCES

1. The investigator should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period (that is, the investigator regularly treats the target population).
2. The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.
3. The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

4. The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.
5. The site should have sufficient processes in place to manage the use of a genetically modified organism containing IMP at the site.
6. The site should have sufficient data management resources to allow prompt data return to the MRC CTU. Sites that have previously participated in MRC CTU-co-ordinated trials should have a proven track record of good data return.

2.2 APPROVAL AND ACTIVATION

Each selected clinical trial site must apply for their local R&D approval. The Principal investigator will be required to sign an Investigator Statement which verifies that the site is willing, and able to comply with the requirements of the trial. In addition and in compliance with the principles of GCP, all site staff participating in the trial must complete the Signature and Delegation of Responsibilities Log and forward this to the RIVER Co-ordinating Centre. The RIVER Co-ordinating Centre must be notified of any changes to trial personnel and/or their responsibilities. An up-to-date copy of this log must be stored in the Trial Master File (TMF) at the site and also at the RIVER Co-ordinating Centre. A clinical trial agreement will also be signed by the NHS Trust or Board. Local laboratory normal ranges must also be provided.

On receipt of all necessary approvals, written confirmation will be sent to the principal investigator. The site's pharmacist will also be informed of the site's activation and an initial drug order will be dispatched to the named pharmacist.

1. The site should conduct the trial in compliance with the protocol as agreed by the Sponsor, by the regulatory authority, and the REC.
2. The principal investigator or delegate should document and explain any deviation from the approved protocol, and communicate this with the trial team at the RIVER Co-ordinating Centre.

2.3 RIVER TRIAL CENTRES

- St Mary's Hospital, Imperial College London
- Guy's and St Thomas' NHS Foundation Trust, London
- Chelsea and Westminster Hospital NHS Foundation Trust, London
- Royal Free London NHS Foundation Trust
- Brighton and Sussex University Hospitals NHS Trust
- Mortimer Market Centre, Central and North West London Foundation Trust

3 SELECTION OF PARTICIPANTS

The eligibility criteria are the standards used to ensure that only medically appropriate participants are considered for this study. Participants not meeting the criteria should not join the study. For the safety of the participants, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other participants with similar diseases, it is important that no exceptions be made to these criteria for enrolment and randomisation to the study.

3.1 ENROLMENT

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

3.1.1 INCLUSION CRITERIA

1. Aged ≥18 to ≤60 years old
2. Able to give informed written consent including consent to long-term follow-up
3. Should be enrolled within a maximum of 4 weeks of a diagnosis of primary HIV-1 infection confirmed by one of the following criteria:
    a. Positive HIV-1 serology within a maximum of 12 weeks of a documented negative HIV-1 serology test result (can include point of care test (POCT))
    b. A positive p24 antigen result and a negative HIV antibody test
    c. Negative antibody test with either detectable HIV RNA or proviral DNA
    d. PHE RITA test algorithm[*] reported as "Incident" confirming the HIV-1 antibody avidity is consistent with recent infection (within the preceding 16 weeks)
    e. Weakly reactive or equivocal 4th generation HIV antibody antigen test
    f. Equivocal or reactive antibody test with <4 bands on western blot
4. Adequate haemoglobin (Hb≥12g/dL for males, ≥11g/dL for females)
5. Weight ≥50kg[†]
6. Willing to start immediate cART and be randomised to continue cART alone or cART plus intervention (HIV vaccines plus HDACi)
7. Willing and able to comply with visit schedule and provide blood sampling

[*] using current cut-offs for optical density as defined by PHE
[†] females aged <20 years of age, and weighing <65kg and <168cm in height will need to have an estimation of blood volume (EBV) >3500mL prior to enrolment . This circumstance is unlikely to arise as most women between the ages of 18 to 20 years would be of child-bearing potential (CBP) and excluded on that basis.

3.1.2 ENROLMENT EXCLUSION CRITERIA

1. Women of child bearing potential (WCBP)[‡]
2. In women with intact ovaries and no uterus, any planned egg donation anytime in the future to a surrogate
3. Intention to donate sperm or father a child within 6 months of the intervention
4. Co-infection with hepatitis B (surface antigen positive or detectable HBV DNA levels in blood) or hepatitis C (HCV RNA positive)
5. Any current or past history of malignancy
6. Concurrent opportunistic infection or other comorbidity or comorbidity likely to occur during the trial e.g.past history of ischaemic or other significant heart disease, malabsorption syndromes, autoimmune disease
7. Any contraindication to receipt of BHIVA recommended combination antiretrovirals
8. Any contraindication to receipt of the strand-transfer integrase inhibitor (INSTI), raltegravir
9. HIV-2 infection
10. Known HTLV-1 co-infection
11. Prior immunisation with any experimental HIV Immunogens (including any component of the vaccines used in the RIVER protocol; simian or human adenoviral vaccine; other experimental HIV vaccines)
12. Current or planned systemic immunosuppressive therapy (inhaled corticosteroids are allowed)
13. Any history of proven thromboembolism (pulmonary embolism or deep vein thrombosis)
14. Any inherited or acquired bleeding diathesis including gastric or duodenal ulcers, varices
15. Concurrent or planned use of any drugs contraindicated with vorinostat i.e. antiarrhythmics; any other drugs that prolong QTc; warfarin, aspirin, sodium valproate
16. Prior intolerance of any of either the components of the vaccine or HDACi,
17. Uncontrolled diabetes mellitus defined as an HBA1C>7%
18. Any congenital or acquired prolongation of the QTc interval, with normal defined as ≤0.40s (≤400ms); bradycardia <55 bpm
19. Participation in any other clinical trial of an experimental agent or any non-interventional study where additional blood draws are required; participation in an observational study is permitted
20. Allergy to egg
21. History of anaphylaxis or severe adverse reaction to vaccines

[‡] defined as any female who has experienced menarche and who has not undergone successful surgical sterilisation (hysterectomy or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhoea ≥ 12 consecutive months; or women on hormone replacement therapy (HRT) with documented serum follicle stimulating hormone (FSH) level > 35mIU/mL); NB bilateral tubal ligation is reversible, a women with only tubal ligation who does not meet any of the other criteria is considered a WCBP

22. Planned receipt of vaccines within 2 weeks of the first trial vaccination administered at PR week 00 (including vaccines such as yellow fever; hepatitis B, influenza)
23. Abnormal blood test results at screening including
    a. Moderate to severe hepatic impairment as defined by Child-Pugh classification
    b. ALT >5xULN
    c. Platelets <150x10^9/L
    d. eGFR <90[§]
    e. uPCR >30 mg/mmol
24. Physical and laboratory test findings: Evidence of organ dysfunction or any clinically significant deviation from normal in physical examination and/or vital signs that the investigator believes is a preclusion from enrolment into the study
25. Active alcohol or substance use that, in the Investigator's opinion, will prevent adequate adherence with study requirements
26. Insufficient venous access that will allow scheduled blood draws as per protocol

3.2 CRITERIA FOR RANDOMISATION

Blood samples will be taken at week 22 (visit window of + 2 weeks) to assess whether the participant can proceed to randomisation. Eligibility must be assessed within 14 days prior to randomisation. Randomisation will take place once the participant meets the following eligibility criteria:

1. Participant is willing to continue on combination antiretroviral therapy
2. HCV PCR negative
3. Undetectable plasma HIV RNA (<50copies/mL or <200copies/mL for the Taqman Roche 2.0 assay).

   Participants who do not have undetectable plasma HIV RNA at week 22 can have this repeated until the HIV RNA falls below the specified threshold. The time period between and the number of repeat tests performed will be at the clinician's discretion and up until virological failure in accordance with standard BHIVA guidelines. If it takes more than 14 days (after week 22) to become undetectable it would be necessary to repeat the whole week 22 visit prior to randomisation.
4. Laboratory parameters[**]
   - Platelet count ≥150x10^9/L
   - eGFR ≥90[§]
   - Adequate haemoglobin (Hb≥12g/dL for males, ≥11g/dL for females)
   - ALT <5xULN
   - uPCR ≤30mg/mmol
   - In diabetics, HbA1C <7%

[§] eGFR is calculated by the local labs using CKD-EPI or MDRD, both equations are acceptable. Units ml/min/1.73m^2.
[**] Results from lab parameters should be within 14 days of randomisation. Repeat tests must be within the ranges as listed above.

5. QTc interval normal, with normal defined as ≤0.40s (≤400ms)
6. Physical examination: No evidence of organ dysfunction or any clinically significant deviation from normal in physical examination and/or vital signs that in that in the opinion of the investigator would be a contraindication to randomisation

3.3 NUMBER OF PARTICIPANTS

The study aims to randomise 52 eligible individuals from 6 UK centres. It is likely that 60 participants will need to be enrolled in order to ensure 52 participants are eligible for randomisation. All participants enrolled in the study and eligible for randomisation will be randomised even if eligible participants exceed the 52 participant target.

Where participants withdraw consent for participation, or are deemed lost to follow-up after randomisation, they will not be replaced. Participants who do not have an undetectable HIV RNA viral load (<50 copies/mL or <200copies/mL for the Taqman Roche 2.0 assay) at week 22 bloods (even after repeat testing) will not be randomised. In such cases, participants will discontinue participation in RIVER. They will be managed as per standard of care (SOC). Raltegravir, as a study drug will no longer be supplied to such patients, although the drug might be continued as part of SOC at the discretion of the treating clinician. Continued participation in the UK seroconverter observational study will be encouraged.

3.4 SCREENING PROCEDURES & PRE-RANDOMISATION INVESTIGATIONS

Eligible individuals will be flagged by a clinical pathway at each clinical site. This is to incorporate one or more of the following:

- Direct referral from participants following recent diagnosis of HIV
- The study will be advertised through NGO, advocacy partners such as THT, HIV ibase, GMFA and through the CHERUB website
- Virology laboratories where recent infection is identified will notify lead clinical principal investigators to follow up with potentially eligible study participants.
- Clinical referrals pathways will be made from colleagues and from other sites where enrolment is not currently planned - through BHIVA, MRC CTU website and clinical networks

Written informed consent to enter into the study and be randomised must be obtained from participants after explanation of the aims, methods, benefits and potential hazards of the study and BEFORE any study-specific procedures are performed or any blood is taken for the study (sample participant information leaflet is available in **Appendix VII**).

It must be made completely and unambiguously clear to the participant that they are free to refuse to participate in all or any aspect of the study, at any time and for any reason, without incurring any penalty or affecting their treatment.

Signed consent forms must be kept by the investigator and documented in the case report form and a copy given to the participant. With consent, a letter should be sent to the general practitioner informing him/her of the study and the participant's involvement in it; unless the participant expresses on the consent form that they do not wish their GP to be informed (sample GP letter is available in **Appendix VI**).

4 SCREENING, ENROLMENT & RANDOMISATION

Eligibility for enrolment and randomisation will be confirmed by a web-based program. At week 0 eligible participants will be enrolled and those eligible after week 22 (or later if repeat visit bloods are required) will be randomised to two parallel groups in a 1:1 ratio (**see Figure 1**). It is likely that most participants will meet all eligibility criteria and be randomised around week 23. Randomisation lists will be computer-generated based on random permuted blocks and will be performed by RIVER Co-ordinating centre staff on receipt of a completed randomisation CRF.

4.1 ENROLMENT/SCREENING (WEEK 0) PRACTICALITIES

Participants must be enrolled into the study **within 4 weeks** of a diagnosis of primary HIV-1 infection. A screening visit will be conducted prior to enrolment where laboratory tests will be conducted in order to determine participant eligibility for enrolment at week 0 (**see Section 6.1.1.A**).

ENROLMENTS
To enrol a participant, fax or email password protected scanned CRFs to the
RIVER Co-ordinating Centre
Monday to Friday 09:00 – 17:00
Fax: +44 (0) 20 7670 4817
mrcctu.river@ucl.ac.uk

4.2 RANDOMISATION PRACTICALITIES

Blood samples will be taken at week 22 to assess whether the participant can proceed to randomisation. As soon as results from the week 22 visit (or later visit if repeat bloods are required) are known and eligibility for randomisation can be determined a completed CRF should be submitted to the RIVER Co-ordinating centre. The result of the randomisation will determine where the participant's first visit post-randomisation will take place (at Royal Free Hospital or at recruiting site).

Further details on the process of randomisation can be found in **Section 9.1.**

RANDOMISATIONS
To randomise, fax or email password protected scanned CRF to the
RIVER Co-ordinating Centre
Monday to Friday 09:00 – 17:00
Fax: +44 (0) 20 7670 4817
mrcctu.river@ucl.ac.uk

A manual randomisation process will be set up to cover any instances when the main electronic system is unavailable. This will be detailed in the study working instructions.

4.3 CO-ENROLMENT GUIDELINES

Participants will not be permitted to co-enrol into other interventional studies for the duration of this study. However there is an expectation that participants will be asked to co-enrol into the UK register of HIV seroconverters (UKR) and other observational studies run by the CHERUB network, this participation is allowable.

5 TREATMENT OF PARTICIPANTS

5.1 IMP MANAGEMENT

All IMPs for the study will be stored and managed by a central pharmacy based at the Royal Free Hospital. They will be responsible for the storage and distribution of IMP to the clinical sites. Raltegravir will be pre-shipped to clinical site pharmacies from where it will be dispensed to enrolled participants. Dispensation of vaccines and vorinostat will take place at the Royal Free Hospital, when participants in Arm B attend for vaccination visits.

Raltegravir will be provided as a marketed product so MHRA-approved clinical trial labelling will be applied at the central pharmacy. Both the vaccines and vorinostat are unlicensed products so MHRA-approved clinical trial labelling will be applied by the manufacturer prior to shipping to RIVER central pharmacy.

Clinical trial IMP will be allocated to a participant on receipt of a completed prescription from the participant's clinician. On no account should any drug assigned to a participant be used by anyone else. Unused drug must be returned to the site pharmacy if a participant withdraws from treatment. The date that each participant started study treatment should be documented in the participant's clinical notes and added to the appropriate CRF.

All drug dispensed at and returned to the site pharmacy should be documented on a site accountability log, maintained by a named person (study pharmacist or research nurse). The designated study pharmacist/nurse will, on receipt of supplies prior to the commencement of the study, conduct an inventory and complete a receipt. Inventories will be conducted monthly and the log returned to the RIVER Co-ordinating Centre. Procedures for drug shipping, labelling, accountability and destruction will be detailed in the RIVER working practice documents. The RIVER Co-ordinating Centre will monitor drug accountability at site visits.

5.2 PRE-RANDOMISATION TREATMENT

Once eligibility for enrolment (Week 0) to the study has been confirmed by the RIVER Co-ordinating centre **all** participants will be initiated on appropriate combination ART (cART). The choice of the exact components of this will be based on HIV genotype, potential drug toxicities and interactions with any other concomitant medication and baseline HIV viral load. Usually at the time of acute HIV seroconversion with high viral load and high rates of skin rash and CNS symptoms, Truvada™ with a boosted Protease Inhibitor (PI) is the preferred regimen. For this study any BHIVA recognised appropriate ART is acceptable with the addition of raltegravir (BID) for the duration of the study, and will be individual participant-clinician choice. In addition to this, the integrase inhibitor (INSTI), raltegravir, will be added for the duration of the study. This has been donated by industry partners for the duration of the study with the plan to simplify treatment at the end of the study to standard triple therapy.

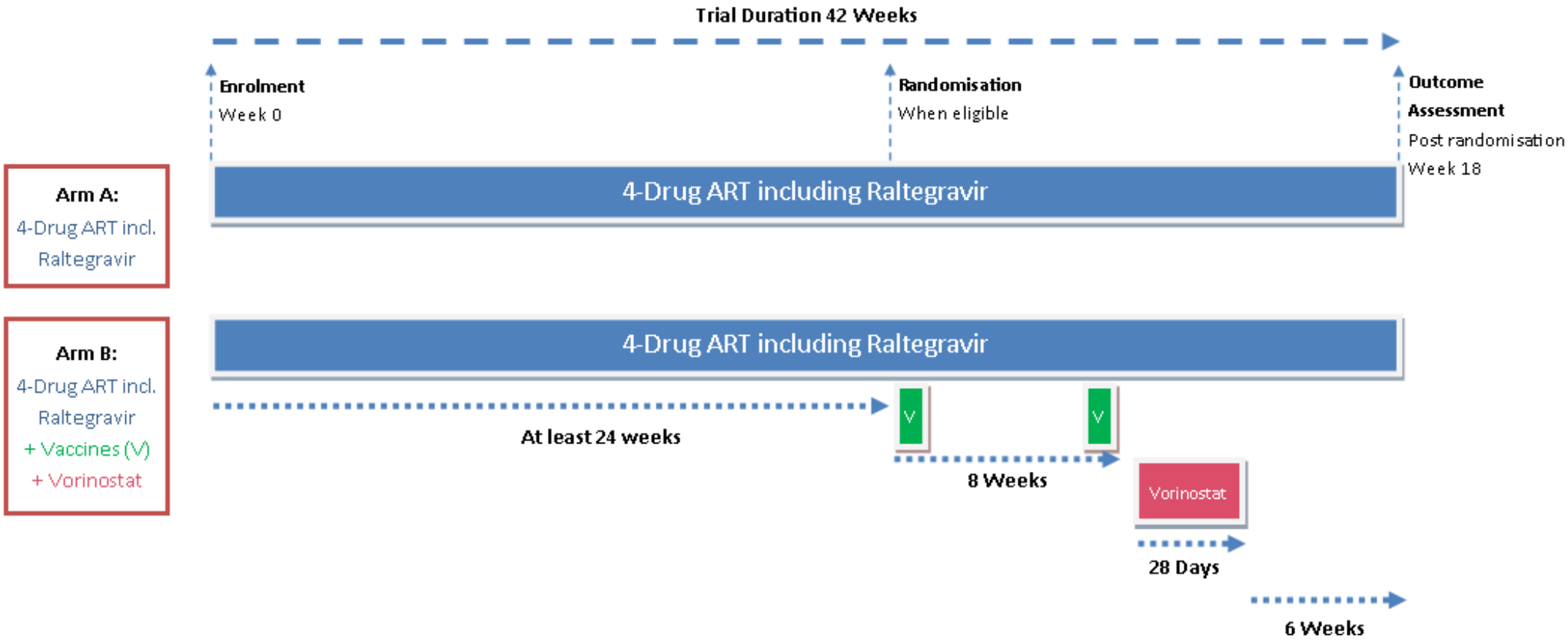
Following safe initiation of ART in accordance with standard BHIVA guidelines, patients who meet the elibility criteria for randomisation (bloods taken at week 22 or at repeat visits **Section 3.2**.) and have confirmed they are happy to proceed, will be randomised. At the randomisation visit, patients will be randomised to one of the two arms i.e. Arm A cART alone or Arm B to receive prime-boost vaccine and HDACi in addition to cART. Participants randomised to Arm B will have their post-randomisation week 00 and week 08 day 1 (PR00 and PR08-1) vaccine visits conducted at the Royal Free Hospital.

5.3 POST-RANDOMISATION (PR) TREATMENT

Once a participant has been randomised to the study the participant allocation will be confirmed by the RIVER Co-ordinating centre. Participants in Arm B will be scheduled for their first vaccination at Royal Free Hospital as per treatment schedule see **Figure 4.**

ARM	COMPONENT	DETAIL
Arm A: 4-Drug ART including raltegravir (cART)	**3-Drug ART (cART)**	Likely consisting of an Nucleoside reverse-transcriptase inhibitor (NRTI) backbone i.e. Truvada® plus a ritonavir-boosted protease inhibitor (PI) e.g. Darunavir + ritonavir. Prescribed at enrolment, week 0, for the duration of the study.
	Raltegravir	***Dosage:*** oral 400mg tablet twice per day (BID). Prescribed at enrolment, week 0, for the duration of the study to end of study, ***SmPC:*** Current version as posted to www.medicines.org.uk ***Marketing Authorisation Holder***: Merck Sharp & Dohme Ltd
Arm B: 4-Drug ART including raltegravir (cART) + Vaccines + vorinostat	**3-Drug ART (cART)**	Likely consisting of an Nucleoside reverse-transcriptase inhibitor (NRTI) backbone i.e. Truvada® plus a ritonavir-boosted protease inhibitor (PI) e.g. Darunavir + ritonavir. Prescribed at enrolment, week 0, for the duration of the study.
	Raltegravir	***Dosage:*** oral 400mg tablet twice per day (BID). Prescribed at enrolment, week 0, for the duration of the study to end of study, ***SmPC:*** Current version as posted to www.medicines.org.uk ***Marketing Authorisation Holder***: Merck Sharp & Dohme Ltd
	ChAdV63.HIVconsv (ChAd)	***Dosage:*** $5x10^{10}$vp ***Administration:*** This dose is obtained by injecting 0.37ml of the vaccine at $1.35x10^{11}$vp/ml without dilution. This prime vaccination is administered intramuscularly (IM) into the deltoid muscle of the non-dominant arm within 1 week of randomisation at visit post-randomisation week 00 (PR00). ***Manufacturer:*** Clinical BioManufacturing Facility (CBF), Oxford, UK ***Storage:*** Vials are stored at between -70 and -90°C
	MVA.HIVconsv (MVA)	***Dosage***: $2x10^{8}$pfu ***Administration:*** This dose is obtained by injecting 0.23 ml of the vaccine IM at $8.6x10^{8}$pfu/ml without dilution. This boost vaccination is administered intramuscularly (IM) into the deltoid muscle of the non-dominant arm at post-randomisation week 8 Day 1 (PR08-1), 2 days prior to start of vorinostat. ***Manufacturer:*** Impfstoffwerk Dessau-Tornau (IDT) Biologika GmbH, Germany ***Storage:*** Vials are stored at between -70 and -90°C
	Vorinostat	**Dosage:** oral 400mg every **third** day from post-randomisation week 8 day 3 (PR08-3) to week 12 day 2 (PR12-2) inclusive. 10 doses in total. ***Administration:*** Vorinostat is administered orally with food. Participants should drink at least 2L fluid/day (on drug dosing days) to prevent dehydration and must report any excessive vomiting or diarrhoea; signs or symptoms of deep vein thrombosis (swelling and pain in a limb, shortness of breath, cough, pleuritic chest pain); unusual bleeding and seek medical attention. ***Manufacturer:*** Merck Sharp & Dohme Ltd ***Storage:*** at 20-25°C, excursions permitted between 15-30°C

Figure 4 RIVER Treatment Schedule



5.4 CRITERIA FOR STARTING VORINOSTAT

Safety assessments will be undertaken to ensure it is safe for participants randomised to Arm B to receive vorinostat at post-randomisation week 08 day 3 (PR08-3). Only participants who meet these criteria will proceed to receive dosing with vorinostat.

Arm B participants must meet the following criteria at post-randomisation week 08 day 1 (PR08-1) in order to commence vorinostat 2 days later at post-randomisation week 08 day 3 (PR08-3):

1. Participant is willing to continue on combination antiretroviral therapy
2. Platelet count ≥150x10^9/L
3. eGFR >90[6]
4. Adequate haemoglobin (Hb≥12g/dL for males, ≥11g/dL for females)
5. ALT <5xULN
6. QTc interval normal, with normal defined as <0.40s (<400 ms)

Any out-of-range results from the above indicate that the participant must not receive vorinostat. Participants in Arm B will then be followed for the rest of the study as per study visit schedule. Analysis of their data will be included as part of the intention to treat analysis of the primary study end point (see **Section 9.5**)

5.5 DISPENSING AND HANDLING

5.5.1 COMBINATION ART (CART)

All participants will be dispensed sufficient supplies of cART to ensure they have sufficient medication to last to the next study visit. Storage of ART is in accordance with the current SmPC for each agent. Suggested dispensing schedules are as follows (but may depend on time taken to meet eligibility criteria for randomisation):

- One-month supply at enrolment (week 0)
- Two-month supply at week 4, and
- Three-month supplies at week 12, randomisation visit and post-randomisation week 12.

5.5.2 RALTEGRAVIR

All participants will be dispensed sufficient supplies of raltegravir to ensure they have sufficient medication to last to the next study visit. Raltegravir is supplied in marketed packs with 60 tablets per bottle. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). Empty and part used bottles/packets of raltegravir should be returned to the study site for adherence and accountability purposes. Suggested dispensing schedule is as above for cART.

[6] eGFR is calculated by the local labs using CKD-EPI or MDRD, both equations are acceptable. Units ml/min/1.73m

5.5.3 VACCINES

Irrespective of clinical site of enrolment, all vaccine study visits for the Arm B participants will be undertaken at one centralised vaccine site (Royal Free Hospital). The reason for this is to ensure uniform temperature and GMO procedures for vaccine procurement, storage, thawing and administration.

The first vaccination (ChAd) takes place at post-randomisation week 00 (PR00), within 1 week after randomisation, and the second vaccine (MVA), 8 weeks later, at PR week 08 day 1 (PR08-1). There is a visit window around the second vaccine of 7 days. On the days of vaccination, the vaccine will be allowed to thaw to room temperature and then administered within 1 hour. The vaccine will be administered intramuscularly (IM) over the deltoid region of the non-dominant arm. The investigator or study nurse will wear gloves and eye protection. During administration of the vaccines, medicines and resuscitation equipment will be immediately available for the management of anaphylaxis. The participant will remain in clinic for ≥1 hour of observation following the receipt of each vaccine. The participant will be contacted by phone by designated study personnel within 72 hrs of receipt of the 1st (ChAd) vaccine. Participants should complete a 3 day vaccine diary following each vaccination. There is a protocol mandated visit 2 days after the second (MVA) vaccination; the participant will be reviewed for AEs relating to the receipt of this second 2nd vaccination at PR week 08 day 3 (PR08-3).

In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, the inoculation sites will be covered with a dressing after immunisation. This should absorb any virus that may leak out through the needle tracks. The dressings will be removed from the injection sites after 30 minutes and disposed of as genetically modified organism (GMO) waste by autoclave and then standard disposal, in accordance with current standard UK practice

5.5.4 VORINOSTAT

Procedures for proper handling and disposal of vorinostat as cytotoxic drug should be employed. The drug should only be dispensed at Royal Free Hospital to those randomised to Arm B. Total number of doses is 10 x 400mg doses over 28 days with 4x100mg capsules taken every third day with food. Vorinostat will be dispensed to participants on PR week 08 day 1 (PR08-1) at the Royal Free Hospital at the participant's second vaccination visit. Participants will be required to bring their vorinostat to their clinic visit (at recruiting site) at PR week 08 day 3 (PR08-3). Participants will be required to take their first vorinostat dose in clinic. Ideally, Day 1 of vorinostat dosing will occur on a Wednesday to avoid study blood draws over the weekend. Dosing should be undertaken at the same time each day. Vorinostat must be kept at room temperature, 15°-30°C (59°-86°F). Vorinostat capsules if damaged or broken should not be used. Vorinostat capsules must not be opened or crushed. If vorinostat powder (within the capsule) comes into contact with skin or mucous membranes, the area must be washed immediately with plain water; participants should notify the site.

5.6 STOPPING DRUG EARLY

5.6.1 COMBINATION ART

Participants ceasing cART at any point on the study for toxicity or other reasons should be managed according to the BHIVA antiretroviral guidelines for adults. Participants choosing to stop cART cannot receive further vaccination or vorinostat if randomised to Arm B.

5.6.2 RALTEGRAVIR

Participants ceasing raltegravir at any point on the study for toxicity or other reasons should be managed according to the BHIVA antiretroviral guidelines for adults. Ideally, an intra-class substitution is preferred i.e. with another integrase inhibitor such as dolutegravir, provided there is no contraindication to use. If, however, it is not possible to substitute another drug to replace raltegravir, they can continue with vaccination and vorinostat as per protocol if randomised to Arm B provided their HIV RNA remains <50copies/mL.

5.6.3 VACCINES

In the event of a severe local or systemic reaction to the first vaccination (ChAd) the following must be considered in regards to administration of the second vaccination (MVA).

1. The second vaccination must not be given if an anaphylactic reaction occurred after the first vaccine administration.
2. In the event of a severe local reaction **or** non-anaphylactic systemic reaction, the site should discuss with the RIVER Co-ordinating Centre team in the first instance who will then discuss with the IDMC for the RIVER study. Advice will be given on whether the second vaccination should be given.

If the participant does not receive the second vaccination for any reason, the participants should remain on cART and in follow-up for the study. Only participants who have received both doses of the vaccines (remain on cART and meet eligibility criteria see **Section 5.4**) can proceed to vorinostat dosing.

5.6.4 VORINOSTAT

In the event of a serious AE related to vorinostat the dosing will be terminated early. See below (**Section 5.7**) and in **Appendix II** for further guidance of toxicity management and dosing.

5.7 DOSE MODIFICATIONS, INTERRUPTIONS & DISCONTINUATIONS

Toxicity will be managed in all randomised groups according to standard clinical practice. Blood tests additional to those described in the study schedule may be requested at any time for clinical management of the participant.

5.7.1 VORINOSTAT TOXICITY MANAGEMENT

In the event of any vorinostat toxicity **Table 3** details actions that should be taken.

Table 3 Toxicity management during vorinostat dosing

Toxicity	Grade	Vorinostat Dosing Action	Continuation
Non-haematological toxicity	Grade 1 or 2	No change unless due to impairment of QoL at discretion of Investigator (consider dose reduction as detailed below for Grade 2 toxicity)	Continue
Non-haematological toxicity	Grade 3 or 4	Cease treatment	Never
Anaemia, neutropenia or thrombocytopenia	Grade 1	No change	Continue
Neutropenia	Grade 2	Withhold vorinostat until returns to Grade ≤1.	If resolves to Grade ≤1, continue vorinostat at full dose. If on rechallenge recurs to Grade 2 or above, cease treatment. G-CSF is allowed at the clinician's discretion
Neutropenia	Grade 3 or 4	Cease treatment	
Febrile neutropenia	Grade 3 or 4	Cease treatment	
Anaemia or thrombocytopenia	Grade 2	Withhold vorinostat until returns to ≤Grade 1	If resolves to ≤ Grade 1, continue vorinostat at full dose. If on rechallenge recurs to Grade 2 or above, cease treatment.
Anaemia or thrombocytopenia	Grade 3 or 4	Cease treatment	

Specific toxicity management in the setting of vorinostat dosing is provided in **Table 3** above. Grade 2 non-haematological toxicities, at the discretion of the treating clinician and with the agreement of the participant, can be managed with dose reduction of vorinostat to 300mg every third day. If the participant is intolerant of this reduced dose then the drug should be ceased all together, see **Section 5.6.**

All attempts must be made to avoid treatment interruptions, especially those for non-medical reasons e.g. 'drug holidays' or lost prescriptions. Treatment interruptions for medical reasons e.g. unable to have oral intake for whatever reason, are unavoidable and are permissible under this protocol as a protocol deviation. Every attempt should be made to limit the duration of treatment interruptions.

5.7.1.A SPECIFIC GUIDELINES FOR MANAGEMENT OF EXPECTED TOXICITIES:

- **Diarrhoea** – management with loperamide is strongly encouraged.
- **Dehydration** – encourage anti-emetics and anti-diarrhoeals as appropriate; encourage drinking of at least 2L fluid per day. Rehydration solutions are also encouraged as appropriate.
- **Nausea and vomiting** – aggressive management is encouraged. Usually responds to 5-HT3 antagonists.
- **Hyperglycaemia** – monitor blood glucose closely, treat with hypoglycaemic agents as appropriate.
- **Electrolyte disturbance** – replace potassium, calcium and magnesium as appropriate, as guided by regular blood electrolyte tests.
- **Venous thromboembolism** – monitor closely for signs and symptoms and investigate if there is suspicion of embolic event or venous thrombosis
- **QTc prolongation** – aggressively manage electrolyte disturbances.
- **Haemotologic toxicities:**
  - **Anaemia** – transfuse as per institutional guidelines. Erythropoietin is allowed on-study as per prescribing guidelines.
  - **Neutropenia** – G-CSF is allowed on-study at investigator discretion.
  - **Thrombocytopaenia -** Platelet transfusions should be administered in the following situations:
    - Platelets <$10x10^9$/L.
    - Platelets <$20x10^9$/L with fever ≥38.5°C due to infection or presumed infection (at discretion of treating clinician).
    - Platelets <$20x10^9$/L with clinically significant bleeding (at discretion of treating clinician).
    - Platelets <$50x10^9$/L in participants having invasive procedures (at discretion of treating clinician).

If the participant is intolerant of the 300mg every third day dose, then the drug should be ceased all together, see **Section 5.6.**

5.7.1.B VIROLOGICAL REBOUND

If a significant increase in viral replication occurs at any time on study (while on or off vorinostat), the Trial Physician will be informed, the participant managed according to current guidelines and continuation in the trial will be managed on a case by case basis. Participants must have HIV RNA <50 copies/mL (<200 copies/mL Taqman Roche 2.0 assay) to be eligible for randomisation.

5.8 OVERDOSE OF TRIAL MEDICATION

All participants should be counselled about the importance of taking the medications as prescribed. It is particularly important that participants understand how many tablets of which type they are taking. Participants must be told to contact their clinic immediately if they take too many tablets. In the event of overdose of study medication, all appropriate medical care will be given and regular tests will be carried out to ensure safety.

5.9 PROTOCOL TREATMENT DISCONTINUATION

In consenting to the study, participants are consenting to study treatment, study follow-up and data collection. However, an individual participant may stop treatment early or be stopped early for any of the following reasons:

- Unacceptable toxicity or adverse event.
- Intercurrent illness that prevents further treatment.
- Development of malignancy.
- Any change in the participant's condition that, in the opinion of the clinician, justifies treatment discontinuation. Clinicians should avoid interrruption of a suppressive ART regimen unless this is unavoidable.
- Inadequate compliance with the protocol treatment in the judgement of the treating physician.
- Withdrawal of consent for study intervention medication by the participant.

As the participant's participation in the study is entirely voluntary, they may choose to discontinue the study treatment at any time without penalty or loss of benefits to which they are otherwise entitled. Although the participant is not required to give a reason for discontinuing their study treatment, a reasonable effort should be made to establish this reason while fully respecting the participant's rights.

Participants should remain in the study for the purpose of follow-up and data analysis (unless the participant withdraws their consent from all stages of the study). If a participant is withdrawn from follow-up, refer to **Section 6.5**.

Data will be kept and included for participants who stop follow-up early.

5.10 ACCOUNTABILITY & UNUSED DRUGS

Investigational Medicinal Product (IMP) will be centrally packed and distributed as described in **Section 5.1.** Supplies of raltegravir will be sent to the hospital pharmacy at each of the participating sites. The hospital pharmacy will document receipt of supplies and returns.

Supplies of raltegravir will be dispensed to participant from the site at which they were enrolled. Vaccines and vorionostat will be dispensed to participants in Arm B at their visits to Royal Free Hospital. Unused vaccine, raltegravir and/or vorinostat will be returned to the study pharmacy and entered onto the accountability log. Vorinostat will be handled and destroyed as per the requirements of a chemotherapy (anti-cancer) drug. Study vaccines will be disposed of as Genetically Modified Organism (GMO) waste by autoclaving and usual disposal, in accordance with current standard UK practice.

IMP accountability will be maintained and monitored by site staff and RIVER Co-ordinating Centre respectively according to the RIVER working practices and pharmacies will be asked to maintain logs of any returned IMP. At the end of the study all non-dispensed IMP will be checked against the inventory before disposal. Disposal of IMP should not be carried without approval of the RIVER Trial Manager and should be carried out according to local pharmacy guidelines and applicable regulations. Documentation of disposal will be provided to RIVER Co-ordinating Centre.

5.11 COMPLIANCE & ADHERENCE

Suitable participant information and fully informed consent procedures will ensure that participants understand the study requirements.

The study vaccines will be given in hospital by clinic nurses intramuscularly therefore few problems with compliance or adherence are envisaged. Compliance and adherence to the cART, raltegravir and vorinostat treatment regimens will be assessed at clinic visits through interview with the participant. Participants will be interviewed about missed doses at study visits, and if they do occur these will be recorded in the CRF.

5.12 TREATMENT DATA COLLECTION

Every drug prescribed, its dose, frequency, route of administration, and duration will be recorded in the study case report forms (CRF).

5.13 CONCOMITANT MEDICATIONS

5.13.1 MEDICATIONS PERMITTED

Antibiotics to treat intercurrent infection, treatment for other co-morbidities not contraindicated for the study e.g. diabetic treatment, hypertension treatment, lipid lowering agents and inhaled corticosteroids are all permitted for concomitant use.

5.13.2 MEDICATIONS NOT PERMITTED

The following medications are not permitted for concomitant use:

- Immunosuppressive drugs (inhaled corticosteroids are permitted).
- Chemotherapy drugs.
- Other HIV vaccines.
- All medication whose co-administration with ritonavir boosted protease inhibitor (e.g. darunavir), raltegravir and NRTI backbone (e.g. Truvada® or Kivexa®) is contraindicated. See details in the relevant SmPC and RIVER study protocol instruction manual (PIM).
- In Arm B drugs that have been associated with Torsades de Pointes and/or are associated with QT shortening (see **Appendix I** and reference www.torsades.org).
- In Arm B, other HDACi i.e. sodium valproate.

5.13.3 MEDICATIONS TO BE USED WITH CAUTION

Intermittent use of non-steroidal anti-inflammatory drugs (NSAIDS), caution is only required if these are given within a week of or during receipt of vorinostat in Arm B participants. The reason for caution is that all NSAIDS can impair platelet function. One of the most common side-effects of vorinostat is thrombocytopenia, and this coupled with impaired platelet function might increase the risk of bleeding.

Co-administration of raltegravir with aluminium and magnesium antacids results in reduced raltegravir plasma levels, separate doses by at least 2hrs to avoid chelation effects on raltegravir and reduced absorption. More information is also provided in **Appendix I**.

6 ASSESSMENTS & FOLLOW-UP

All randomised participants will be asked to attend follow-up visits until week 18 after randomisation (PR18) in order to assess primary outcome measures. Participants will also be invited to an annual follow-up visit at the site for 5 years following the end of the interventional part of the study.

Staff recording clinical data will be identified by each site Principal Investigator, receive appropriate training and sign a delegation Log. Clinical data will be obtained through consultation with the participant, their medical team, or their medical records. Laboratory measures will be extracted from participant notes/electronic records; study nurses will administer the QOL questionnaires to participants. All data will be recorded on paper CRFs submitted to the RIVER Co-ordinating Centre for processing within 7 days of the study visit.

Any additional visits or diagnostic/laboratory tests needed for participant management should occur as required at the discretion of the treating physician.

6.1 TRIAL ASSESSMENT SCHEDULE

Procedures planned at study visits follow the schedule outlined in **Table 1**. Participants will be provided with a summary of what to expect at study visits prior to providing consent, to ensure they are fully informed of the commitment required as part of the RIVER study. Details of logistics surrounding study visits will be included in study specific working practices and training for study staff will be provided.

6.1.1.A SCREENING AND ENROLMENT (WEEK 0)

One of the eligibility criteria for enrolment in to the RIVER study is a confirmed HIV diagnosis within 4 weeks of enrolment. Therefore potentially eligible individuals must undergo all tests required for assessment of eligibility in the 4 weeks following diagnosis. All results from this screening must be available for enrolment (Week 0). All participants must be given adequate information about the study together with a Participant Information Leaflet (**Appendix VII**) and be given an opportunity to ask questions about the study. Study staff will be asked to maintain a log of participants who are not eligible or do not agree to take part in the study.

Study staff will ascertain that the information leaflet has been fully understood and obtain written informed consent before any study procedures are carried out. All individuals screened must have their name, date of birth and study number recorded in the Trial Register and have appropriate CRFs completed. The register must be stored by the investigator in a secure place only accessible to appropriate clinical staff. If an individual is not subsequently randomised the reason should be recorded in the register.

There will be a clinic interview, during which baseline demographics, clinical and behavioural data relevant to the eligibility criteria in **Section 3.1.1** and **Section 3.1.2** will be collected onto a CRF.

Screening will include:

1. Recording the clinical history, including HIV test results (Resistance test & Hepatitis B (sAg, sAb, Core Ab) and C serology (HCV Ab and HCV PCR).
2. Recording specific clinical details to ensure female candidates are not women of CBP.
3. Physical examination (including height, weight and blood pressure).
4. Resting 12-lead ECG.
5. If results are not already available, routine blood will be taken for:
    a. Full blood count (haemoglobin, haematocrit, white blood cell count (WBC) with differential and platelets).
    b. Biochemistry tests (ALT, AST, alkaline phosphatase, total bilirubin, albumin, eGFR, creatinine kinase).
    c. Urine Protein Creatinine Ratio (uPCR).
    d. Lipids (total cholesterol, LDL, HDL, triglycerides).
    e. T lymphocyte subsets (total lymphocytes, percent and/or absolute CD3/CD4/CD8).
    f. Viral load measurements.
    g. HbA1C (only if known diabetes mellitus).

Results of the clinical history and evaluations are to be entered on a case report form. Once clinic staff are satisfied that the participant is eligible for the study a completed screening CRF should be submitted to the RIVER Co-ordinating centre for verification. A confirmation of enrolment will be sent to the site by email and also relayed over the telephone. Once this confirmation has been received the participant should be commenced on cART treatment as outlined in **Section 5.5**. Eligibility for randomisation will be assessed at Week 22. Participants will not be randomised until all eligibility criteria have been met, see **Section 3.2**, at which time the clinic staff will be informed which arm the participant have been allocated to. All participants up until post-randomisation week 00 (PR00) will receive the same treatment.

6.2 PROCEDURES FOR ASSESSING EFFICACY

The efficacy of cART will be determined using viral load assays obtained at the local laboratories. In particular participants must have a plasma HIV RNA <50 copies/mL (<200 copies/mL if Taqman 2.0 Roche testing platform used) to be eligible for randomisation, either at week 22, or at any necessary repeat visits. Any evidence of viral rebound during the study must be managed in accordance with standard national treatment guidelines.

6.3 PROCEDURES FOR ASSESSING SAFETY

All standard routine toxicity testing for cART, vaccine and vorinostat will be made according to the study visits. In addition in the event of any abnormal blood test results these will be followed up with good clinical practice accordingly. Specific monitoring of the following parameters i.e. FBC, renal function (eGFR and uPCR), electrolytes i.e. calcium, magnesium and potassium; LFTs and blood glucose will be conducted at specific time points in the study commensurate with the vorinostat dosing schedule Arm B.

Adverse events will be reported on the case report form. Adverse events (clinical and laboratory) will be graded using the 2004 (amended 2009) Division of AIDS toxicity grading scale (see **Appendix III**).

Serious adverse events will be defined according to principles of GCP, and should be reported to the CTU within 24 hours of the site being aware (see **Section 7**). All adverse events, after randomisation, meeting the definitions above should be reported on study CRFs, regardless of their relationship to HIV. Prior to randomisation only grade 3 and 4 adverse events and Serious Adverse Events (SAE) are collected.

6.4 PROCEDURES FOR ASSESSING QUALITY OF LIFE

Participants will be asked to complete the EQ-5D-5L questionnaire at enrolment, randomisation, post-randomisation week 08 day 3 (PR08-3) and the final visit, post-randomisation week 18 (PR18). (**Appendix X**).

6.5 EARLY STOPPING OF FOLLOW-UP

If a participant chooses to discontinue their study treatment, they should always be followed up providing they are willing, that is, they should be encouraged to not leave the whole study; if they do not wish to remain on study follow-up, however, their decision must be respected and the participant will be withdrawn from the study completely. The RIVER Co-ordinating centre should be informed of this in writing using the appropriate documentation. Participants withdrawing completely have a negative impact on a study's data.

If the medical data collected during the participant's participation in the study are kept for research and analysis purposes, they can be anonymised if necessary. Consent for future use of stored samples already collected can be refused when leaving the study early (but this should be discouraged and should follow a discussion).

All participants will be encouraged to enrol in the UK seroconverters register and will also be followed up in the long-term as part of the RIVER study (see **Section 6.9**), which may include flagging with the NHS Information Centre, ONS or similar approaches.

6.6 PARTICIPANT TRANSFERS

If a participant moves from the area, every effort should be made for the participant to be seen at another participating study site. A copy of the participant's CRFs should be provided to the new site and the participant will need to sign a new consent form. Once this has been done, the new site will take over responsibility for the participant; until this has been done, responsibility for the participant lies with the original site.

6.7 LOSS TO FOLLOW-UP

In the statistical analysis, a patient will be classified as 'lost to follow-up' if they have not been seen in clinic for more than 3 months. For operational management, a patient will be classified as 'lost to follow-up' (i.e no further efforts to trace the patient are being made) when they have missed 3 scheduled clinic visits and despite at least 3 documented attempts to contact them. Provided patients have consented to the site contacting their GP, the site will attempt to find further

information on the patient's clinical status via the GP. Subsequently, if the patient attends clinic and a CRF is received by the RIVER Co-ordinating Centre, the 'lost to follow-up' status will be reversed.

6.8 ASSESSMENTS AT TRIAL CLOSURE

At the final study visit (PR18) all blood assessments will be taken for the final study end point. The participant will then continue with ART triple therapy and return to standard clinical care in consultation with their doctor.

6.9 LONG-TERM FOLLOW-UP

All participants will be followed-up annually for 5 years following the end of the interventional phase of the study.

To gather specific safety information specifically in regards to vorinostat – as the mechanism of action of vorinostat is gene modification this may carry unforeseen long-term consequences beyond the timeframe of the main study. Specific questions asked will include:

1. Safety related
   - Any new or exacerbation of haematological conditions including lab abnormalities.
   - Any new malignancies or pre-malignancies including skin cancers, anal intraepithelial neoplasia (AIN), cervical intraepithelial neoplasia (CIN), Barrett's oesophagus etc.
   - Any new or exacerbation of pre-existing medical problems.
   - Outcome of any pregnancies in female partners.
2. To gather HIV related information:
   - Antiretroviral drug use.
   - T-cells and viral load.
   - AIDS-defining illness (ADI) or serious non-AIDS (SNA) events.
3. To obtain longer-term follow up on impact of the intervention on measures of viral reservoir beyond the scope of the current research study a 40mL blood draw for HIV reservoir assessment (DNA) and gene transcription studies will be collected at each annual visit.

7 SAFETY REPORTING

The principles of GCP require that both investigators and sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical studies. These procedures are described in this section of the protocol. Section 7.1 lists definitions, Section 7.3 gives details of the investigator responsibilities and Section 7.4 provides information on the RIVER Co-ordinating Centre responsibilities.

7.1 DEFINITIONS

The definitions of the EU Directive 2001/20/EC Article 2 based on the principles of GCP apply to this study protocol. These definitions are given in Table 4.

Table 4 Definitions

TERM	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical study subject to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SmPC) or Investigator Brochure (IB) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: ▪ Results in death ▪ Is life-threatening[‡‡] ▪ Requires hospitalisation or prolongation of existing hospitalisation[§§] ▪ Results in persistent or significant disability or incapacity ▪ Consists of a congenital anomaly or birth defect ▪ Is another important medical condition[***]

[‡‡] The term life-threatening in the definition of a serious event refers to an event in which the participant is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

[§§] Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, that has not worsened or for an elective procedure do not constitute an SAE.

[***] Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation or development of drug dependency.

7.1.1 MEDICINAL PRODUCTS

An investigational medicinal product is defined as the tested investigational medicinal product and the comparators used in the study. (EU guidance ENTR/CT 3, April 2006 revision). In the RIVER study this includes:

- Vorinostat
- Raltegravir
- Combination ART backbone treatment
- ChAdV63.HIVconsv.
- MVA.HIVconsv vaccine

7.1.2 ADVERSE EVENTS

Adverse Events include:

- An exacerbation of a pre-existing illness.
- An increase in frequency or intensity of a pre-existing episodic event or condition.
- A condition (even though it may have been present prior to the start of the study) detected after study drug administration.
- Continuous persistent disease or a symptom present at baseline that worsens following administration of the study treatment.

Adverse Events do not include:

- Medical or surgical procedures; the condition that leads to the procedure is the adverse event.
- Pre-existing disease or a condition present before treatment that does not worsen.
- Hospitalisations where no untoward or unintended response has occurred, e.g., elective cosmetic surgery, social admissions.
- Accidental overdose of medication without signs or symptoms.

7.1.3 DISEASE-RELATED EVENTS

All adverse events meeting the definitions above, regardless of their relationship to HIV and should be graded **(see Appendix III).** For AE reporting requirements refer to **Section 7.4.**

In particular, all deaths should be reported as fatal SAEs.

7.2 OTHER STUDY-SPECIFIC REQUIREMENTS & EXPLORATORY OUTCOMES

7.2.1 LARGE VOLUME BLOOD DRAW

The total blood draw over the 42 weeks of study is approximately 1500 ml. The largest blood draws occur at randomisation (390ml) and post-randomisation week 16, PR16 (384ml). The UK blood transfusion service (www.blood.co.uk/giving-blood) recommends the following in regards to donating blood:

1. Aged 17-65 years and generally in good health.
2. Male donors can donate 4 times in a 12 month period, with each blood donation equal to 470 ml i.e. a total of 1880 ml/annum.
3. Female donors can donate 470mLs every 16 weeks, this is to reduce the risk of iron deficiency anaemia in those still menstruating.

4. Weight must be at least 50 kg, but in females under 20 years of age and weighing <65kg and less than 168cm in height, blood volume needs to be estimated before donation.

In the RIVER protocol, the risks of the blood draws are minimised through the following:

1. Haemoglobin (Hb) within the normal range and weight ≥50kg as inclusion criteria for participation; women of CBP are excluded from participation, and none of the women included will be menstruating.
2. Participants have primary HIV infection and it is most unlikely that there will be any impact on the haematological axis in such participants.
3. Hb checks prior to the larger volume blood draws at randomisation and PR16 i.e. at week 22 and PR12 respectively.

7.2.2 EXPLORATORY OUTCOMES – HUMAN ENDOGENOUS RETROVIRUSES

Some human endogenous retroviruses (HERVs) types have been associated with disease states including lymphoma and multiple sclerosis but the exact role (if any) that HERVs play in their aetiology remains to be determined. The effect of vorinostat on human endogenous retroviruses (HERVs) is unknown. Any elevation of HERVS that might occur as a consequence of exposure to vorinostat is unlikely to be sustained, as exposure to vorinostat is restricted to 10 doses only. Information on incident cancers including lymphoma will be collected for the duration of the study and 5 years post (see **Section 6.9**). The levels of these viruses may be measured under two circumstances, first if an excess of malignancies in which HERVS could be plausibly be involved is seen; or second, as an additional exploratory analysis.

7.3 OTHER NOTABLE EVENTS

There are several other notable events which if they occur during the study should be recorded in the participant's medical notes and reported to the RIVER Co-ordinating centre. Notable events may also be reportable as an SAE if any of the criteria listed in **Table 4** are met. Refer to **Appendix IV** for a full list of notable events.

7.3.1 VACCINE RELATED EVENTS

GSK, as owners of the ChAd vector, have a list of vaccine related adverse events which are of specific interest to them. Information on notable events will be collected on a study CRF, and further information on the event may be requested.

7.3.2 CANCERS

Despite the fact that vorinostat is an HDACi licensed in the USA for the treatment of cutaneous T-cell lymphoma and multiple myeloma, in regards to the non-clinical toxicology of the agent, carcinogenicity studies have not been performed. However, vorinostat is mutagenic in vitro in the bacterial reverse mutation assays causing chromosomal aberrations in vitro in Chinese hamster ovary and increased incidence of micro-nucleated erythrocytes when administered to mice (mouse micronucleus assay). The risk of malignancy in participants exposed to vorinostat, even for short periods, is unknown.

For this reason any diagnosis of cancer (or worsening cancer) throughout the study should be reported on a cancer specific study CRF.

Information on any new malignancies will also be collected during long-term follow up, where participants will be followed up annually for 5 years following the completion of the intervention phase of the study (see **Section 6.9**).

7.3.3 PREGNANCY

Women of child-bearing potential cannot participate in this study. In pre-clinical studies, there were chromosomal abnormalities induced by vorinostat in hamster ovary lines. It is unknown what impact, short exposure to vorinostat might have on the human ovary. Should the participant in the study father a child within 1 year of completing the course of vorinostat consent from the mother of the child will be sought to allow follow-up to the birth of the child.

Pregnancy in a partner should be reported as a notable event.

7.4 INVESTIGATOR RESPONSIBILITIES

All non-serious AEs and ARs, whether expected or not, should be recorded in the participant's medical notes and dependent on what stage of the study the participant has reached may need to be reported to the RIVER Co-ordinating centre:

- Prior to randomisation, when the participant is on cART alone, only grade 3 and 4 adverse events need to be reported on study CRFs.
- From randomisation up until the participant's last study visit (PR18), in addition to grade 3 or 4 events, **all** non-serious AEs and ARs, whether expected or not, should also be recorded in the participant's medical notes and reported on the relevant study CRF.
- Adverse events should be sent to the RIVER Co-ordinating centre within the agreed timescale of **7 days** from awareness of the event.
- SAEs, SARs and Notable Events should be notified to the RIVER Co-ordinating Centre within **24 hours** of the investigator becoming aware of the event.

7.4.1 INVESTIGATOR ASSESSMENT

7.4.1.A SERIOUSNESS

When an AE or AR occurs, the investigator responsible for the care of the participant must first assess whether or not the event is serious using the definition given in **Section 7.1.** If the event is serious, then an SAE Form must be completed and the RIVER Co-ordinating Centre notified within 24 hours.

7.4.1.B SEVERITY OR GRADING OF ADVERSE EVENTS

The severity of all AEs and/or ARs (serious and non-serious) in this study should be graded using the toxicity grading table in **Appendix III.**

7.4.1.C CAUSALITY

The investigator must assess the causality of all serious events or reactions in relation to the study therapy using the definitions in **Table 5**. There are five categories: unrelated, unlikely, possible, probable, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possible, probable or definitely related, then the event is classified as an SAR.

If an SAE is considered to be related to study treatment and drug is stopped or the dose modified, refer to **Section 5.7**.

Table 5 Assigning Type of SAE Through Causality

RELATIONSHIP	DESCRIPTION	SAE TYPE
Unrelated	There is no evidence of any causal relationship	Unrelated SAE
Unlikely	There is little evidence to suggest that there is a causal relationship (for example, the event did not occur within a reasonable time after administration of the study medication). There is another reasonable explanation for the event (for example, the participant's clinical condition, other concomitant treatment).	Unrelated SAE
Possible	There is some evidence to suggest a causal relationship (for example, because the event occurs within a reasonable time after administration of the study medication). However, the influence of other factors may have contributed to the event (for example, the participant's clinical condition or other concomitant treatments).	SAR
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	SAR
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	SAR

7.4.1.D EXPECTEDNESS

If there is at least a possible involvement of the study treatment (or comparator), the investigator must assess the expectedness of the event. An unexpected adverse reaction is one not previously reported in the current Summary of Product Characteristics (SmPC) or one that is more frequent or more severe than previously reported. The definition of an unexpected adverse reaction (UAR) is given in **Table 4.** If a SAR is assessed as being unexpected, it becomes a SUSAR.

Please see **Appendix II** for details of expected toxicities associated with the drugs being used in this study.

7.4.1.E NOTIFICATION

The RIVER Co-ordinating Centre should be notified by the investigator of all SAEs within **24 hours** of the investigator becoming aware of the event.

Investigators should notify the RIVER Co-ordinating Centre of any SAEs occurring from the time of enrolment until 12 weeks after the participant's last study visit (up until post-randomisation week 30). Any subsequent events that may be attributed to treatment should be reported to the MHRA using the yellow card system.

Having reviewed and checked SAEs, the RIVER Co-ordinating Centre will then be responsible for reporting these to the sponsor within 7 days.

7.4.2 NOTIFICATION PROCEDURE

1. The SAE Form must be completed by an investigator (a consultant named on the Signature List and Delegation of Responsibilities Log who is responsible for the participant's care), with due care being paid to the grading, causality and expectedness of the event as outlined above. In the absence of the responsible investigator, the form should be completed and signed by a member of the site study team and faxed or emailed as appropriate. The responsible investigator should subsequently check the SAE Form, make changes as appropriate, sign and then re-fax to the RIVER Co-ordinating centre as soon as possible. The initial report must be followed by detailed, written reports as appropriate.

   The minimum criteria required for reporting an SAE are the study number, name of investigator reporting, the event, and why it is considered serious.

2. The SAE Form must be sent by fax to the RIVER Co-ordinating Centre Fax: +44 (0) 20 7670 4817 or password-protected email to mrcctu.river@ucl.ac.uk

3. Follow-up: participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline, or until the event has stabilised. Follow-up should continue after completion of protocol treatment if necessary. A further SAE Form, indicated as 'Follow-up' should be completed and faxed to the RIVER Co-ordinating Centre as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The participant must be identified by study number, 3 letter code and year of birth only. The participant's name should not be used on any correspondence and should be deleted from any test results.

4. Staff should follow their institution's procedure for local notification requirements.

SAE REPORTING
Within 24 hours of becoming aware of an SAE, please fax a completed SAE form to the RIVER Co-ordinating Centre on:
Fax: +44 (0) 20 7670 4817 or email information to mrcctu.river@ucl.ac.uk

7.5 RIVER CO-ORDINATING CENTRE RESPONSIBILITIES

Medically-qualified staff at the MRC CTU (RIVER co-ordinating centre) and/or the Chief Investigator (or a medically-qualified delegate) will review all SAE reports received. The causality assessment given by the local investigator at the hospital cannot be overruled; in the case of disagreement, both opinions will be provided in any subsequent reports.

The sponsor has delegated responsibility to MRC CTU who will be responsible for the reporting of SUSARs and other SARs to the regulatory authority (MHRA) and the research ethics committee, as appropriate. Fatal and life-threatening SUSARs must be reported to the competent authority within 7 days of the RIVER Co-ordinating Centre becoming aware of the event; other SUSARs must be reported within 15 days.

The RIVER Co-ordinating Centre will also keep all investigators informed of any safety issues that arise during the course of the study and will submit Development Safety Update Reports (DSUR) to Competent Authorities (Regulatory Authority and Ethics Committee) and provide to drug companies as necessary.

Any drug companies involved (MSD and GSK) will also be notified of all reportable (serious and unexpected and drug-related/unknown relationship) events. The RIVER Co-ordinating Centre will also provide companies with a copy of the Annual Safety Report.

8 QUALITY ASSURANCE & CONTROL

8.1 RISK ASSESSMENT

The Quality Assurance (QA) and Quality Control (QC) considerations have been based on a formal Risk Assessment, which acknowledges the risks associated with the conduct of the study and how to address them with QA and QC processes. QA includes all the planned and systematic actions established to ensure the study is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the study-related activities are fulfilled. This Risk Assessment has been reviewed by the Research Governance Committee (RGC) and has led to the development of a Quality Management Plan (QMP), which will be kept separately.

8.2 CENTRAL MONITORING AT THE RIVER CO-ORDINATING CENTRE

Data will be entered at each site onto Case Report Forms (CRFs). These CRFs will be checked at RIVER Co-ordinating Centre for missing or unusual values (range checks) and checked for consistency within participants over time. If any such problems are identified, the site will be contacted and asked to verify or correct the entries made on the CRFs. The RIVER Co-ordinating Centre will also send reminders for any overdue and/or missing data with regular inconsistency reports of errors.

Other essential study issues, events and outputs will be detailed in the Data Management, Monitoring and Quality Management Plans that are based on the study-specific Risk Assessment.

8.3 ON-SITE MONITORING

Staff from MRC CTU will visit clinical sites, central pharmacy and laboratories to validate and monitor data and procedures. The frequency, type and intensity of routine monitoring and the requirements for triggered monitoring will be risk based the details of which will be documented in a study Monitoring Plan. This plan will also detail the procedures for review and sign-off.

A site initiation visit with training will be performed at each study site by staff from the MRC CTU. The site initiation visits will include training in the administration of study drug, as well as the study procedures. All laboratories will be required to provide evidence of certification before site initiation. The monitoring will adhere to the principles of GCP and the Monitoring Plan.

Monitors will:

- Verify completeness of the Investigator Site File.
- Confirm adherence to protocol.
- Review eligibility verification and consent procedures.
- Look for missed clinical event reporting.
- Verify completeness, consistency and accuracy of data being entered on CRFs.
- Evaluate drug accountability.
- Provide additional training as needed.

The monitors will require access to all participant medical records including, but not limited to, laboratory test results and prescriptions. The investigator (or delegated deputy) should work with the monitor to ensure that any problems detected are resolved.

8.3.1 Direct Access to Participant Records

Participating investigators should agree to allow study-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data and documents as required. Participants' consent for this must be obtained. Such information will be treated as strictly confidential and will in no circumstances be made publicly available.

The following data should be verifiable from source documents:

- All signed consent forms
- Dates of assessments including dates specimens were taken and processed in the laboratory
- Eligibility and baseline values for all participants
- All clinical endpoints
- All serious/severe adverse events
- Routine participant clinical and laboratory data
- Drug compliance
- Dates drug dispensed and (if necessary) drugs returned
- Pharmacy/clinic drug logs
- Concomitant medication.

8.3.2 Confidentiality

The investigator must ensure that participants' anonymity will be maintained and that their identities are protected from unauthorised parties. Participants will be assigned a study identification number and this will be used on CRFs; participants will not be identified by their name. The investigator will keep securely a participant study register showing identification numbers, surnames and date of birth. This unique study number will identify all laboratory specimens, case report forms, and other records and no names will be used, in order to maintain confidentiality, following the principles of the UK DPA. All records will be kept in locked locations for 15 years after the end of the study (**Section 11.1**). Clinical information will not be released without written permission, except as necessary for monitoring, auditing and inspection purposes.

9 STATISTICAL CONSIDERATIONS

9.1 METHOD OF RANDOMISATION

Participants will be randomised centrally using a computer-generated algorithm based on random permuted blocks (practical details will be detailed in the Statistical Master File).

9.2 OUTCOME MEASURES

Analyses will compare the two arms on the following outcome measures:

9.2.1 PRIMARY OUTCOME MEASURE

The primary outcome measure is total HIV DNA from CD4 T-cells averaged across post- randomisation weeks 16 and 18.

9.2.2 SECONDARY OUTCOME MEASURES

Secondary outcome measures include

- Clinical and laboratory adverse events
- Further assessment of the HIV reservoir e.g. HIV integrated DNA; HIV cell associated RNA; plasma HIV RNA measured with an ultra-low copy assay i.e. with a threshold of <1 copy/ml and viral outgrowth assays.
- Studies of immune function including measuring the latently-infected resting memory T-cells and cytotoxic immune responses
- Changes in inflammatory biomarkers

9.3 SAMPLE SIZE

The sample size calculation is based on the primary endpoint which will be analysed on a $\log_{10}$-scale. A total number of 52 individuals will be randomised based on the following assumptions:

- The combination intervention will confer a 50% reduction in the primary outcome measure when compared with cART alone. On a $\log_{10}$-scale this corresponds to a difference between the two arms of 0.30103.
- Standard deviation (SD) is 0.4 for a single measurement in both arms based on two publications:
  - Reported on data from 31 participants treated with HAART at PHI in the French PRIMO study. The median (IQR) at 6 and 12 months after starting treatment were 2.30 (2.10, 2.70) and 2.10 (1.80, 2.40) $\log_{10}$ DNA copies/10^6 PBMC respectively suggesting a SD of 0.45 at both time points[47].
  - Reported on 8 participants with PHI. At week 52, the median (IQR) was 494 (250 - 694) for total HIV-1 DNA copies/10^6 CD4+ T-cells suggesting a SD (in log scale) of 0.33[48].
- 1:1 allocation of participants in the two study arms.
- Method of analysis: comparing the treatment arms in terms of absolute HIV total DNA level at post-randomisation weeks 16 and 18 adjusted for baseline (here: randomisation) level

(analysis of covariance; ANCOVA); one baseline measurement and two follow-up measurements (at PR16 and 18) will be taken for an individual participant.

- A correlation coefficient of 0.5 between a baseline measurement and a PR 16/18 measurement, and a correlation coefficient of 0.7 for measurements at PR 16 and 18.
- Two-sided $\alpha = 0.05$ for the null hypothesis that there is no difference between the two arms in the primary endpoint.

Under the above assumptions, a sample size of 52 individuals would provide 94% power to detect a 50% reduction in HIV total DNA (44 and 36 participants would provide 90% and 83% power, respectively).

9.4 INTERIM MONITORING & ANALYSES

An Independent Data Monitoring Committee (IDMC) will monitor the study, an IDMC Charter will be drawn up that describes the membership of the IDMC, relationships with other committees, terms of reference, decision-making processes, and the timing and frequency of interim analyses.

The IDMC will meet once before the start of the study, and approximately at 6-month intervals after start of enrolment. In addition, an IDMC meeting may be requested by IDMC members, the Chief Investigator or the TMG at any time

9.5 ANALYSIS PLAN (BRIEF)

The analyses will be described in detail in a full Statistical Analysis Plan. This section summarises the main issues.

HIV total DNA from CD4 T-cells average at post-randomisation weeks 16 and 18 (PR16 and PR18) will be compared between the two groups as allocated (intention to treat, ITT) by analysis of covariance with adjustment for values at randomisation. The analysis will be performed on all eligible participants randomised. An imputation method will be used to estimate missing HIV total DNA values in participants with unobserved values and this will be defined in the Statistical Analysis Plan.

The frequency of grade 3 and 4 adverse events will be tabulated by body systems and randomised group and the groups will be compared using Fisher's exact test.

10 PARTICIPANT AND PUBLIC INVOLVEMENT

A representation from people living with HIV sits on the RIVER Trial Management Group and has been closely involved in the development of the protocol. In addition there is another patient representative on the Trial Steering Committee (**Appendix V**) and has provided input on the protocol and participant information leaflets. This representative also forms, with others, a community advisory board (CAB) for the CHERUB collaboration who will help disseminate the study's results beyond the academic and healthcare professional community to other participant groups and the wider public.

11 REGULATORY & ETHICAL ISSUES

All regulatory requirements (including safety reporting, **Section 7** and below) will be met by the sponsor or their delegated authorities. We will follow the statutory requirements for consent as set out in the Medicines for Human Use (Clinical Trials) regulations.

11.1 COMPLIANCE

The end of the interventional phase of the study is defined as being 12 weeks after each participant's last visit and investigators should continue to notify the RIVER Co-ordinating centre of all SAEs for this period. After this time the participant's long-term follow-up phase will commence (**Section 6.9**). The study will end after the last participant completes their 5th annual visit.

11.1.1 REGULATORY COMPLIANCE

The study complies with the principles of the Declaration of Helsinki (1996).

It will also be conducted in compliance with the approved protocol, the principles of Good Clinical Practice (GCP) as laid down by the Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 (The Medicines for Human Use [Clinical Trials] Regulations 2004) and subsequent amendments, the UK Data Protection Act (DPA number: Z6364106), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

11.1.2 SITE COMPLIANCE

All sites will comply with the above. An agreement will be in place between the site and the sponsor, Imperial College London, setting out respective roles and responsibilities.

The site will inform the Trials Unit as soon as they are aware of a possible serious breach of compliance, so that the Trials Unit can report this breach if necessary within 7 days as per the UK regulatory requirements. For the purposes of this regulation, a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects in the study, or
- The scientific value of the study

11.1.3 DATA COLLECTION & RETENTION

CRFs, clinical notes and administrative documentation should be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for 15 years after the end of the study. During this period, all data should be accessible to the competent or equivalent authorities, the Sponsor with suitable notice. The data may be subject to an audit by the competent authorities.

11.2 ETHICAL CONDUCT OF THE STUDY

Written, informed consent will be obtained from all participants before enrolment by the site principal investigator or an appropriately trained Consultant, Specialist Registrar, or Research Nurse

(as delegated by the Principal Investigator). Participants will be free to withdraw from the study at any time, and this will be explicitly stated in participant information leaflets.

11.2.1 Ethical Considerations

The success of current ART has converted HIV from a life-threatening disease into a chronic illness that does not significantly impair life-span compared to HIV infected counterparts. Importantly, the START study[49] was stopped early, in May 2015, as there was a clear clinical benefit to starting ART with high CD4+ T-cell count (including above 500 cells/mm^3) compared to deferring to the current threshold of 350 cells/mm^3. It is likely that all guidelines, including BHIVA will be amended to reflect this definitive data in support of universal treatment. However, despite these findings, there are potential limitations of lifelong ART, i.e. the need for adherence to oral daily ART with potential toxicities, issues with life-long adherence, risk of drug resistance and high long-term cost burden to the health services. Considering the START study findings and it's likely impact on all ART guidelines, the use of ART among individuals with PHI, will not present a substantial ethical issue even though the current BHIVA guidelines for the health of the patient, only recommend ART treatment of PHI if there is neurological disease, AIDS, a CD4+ <350 cells/mm^3 or a severe seroconversion. We anticipate the BHIVA guidelines will shortly be amended later this year to incorporate the findings of the START trial. BHIVA guidelines also support the use of 'treatment as prevention', this involves the use of ART in an HIV-infected individual to reduce HIV transmission to uninfected partner(s).

There is an unknown long-term risk of toxicity following use of vorinostat. The recent presentation is the first multiple dosing data for HIV+ individuals and has been shown to be safe and well tolerated in the short-term – follow-up of over a year – with only minimal side effects[8].

The vaccine strategy has been given to HIV uninfected individuals and is safe and well tolerated. Further data on the safety of the vaccines used in the RIVER study, will be available prior to ethics submission, from the ongoing IrsiCaixa study being conducted in in patients with PHI on ART[41].

Participants will be given a small sum of money towards the cost of their travel to their clinic. For Arm B patients only, reasonable costs for return travel to the vaccination centre located at the Royal Free Hospital (study visit at PR 00 and 08) will be reimbursed.

11.2.2 Ethical Approvals

Before initiation of the study at each clinical site, the protocol, all informed consent forms, and information materials to be given to the prospective participant will be submitted to ethics committee for approval. Any further amendments will be submitted and approved by the designated ethics committee.

The rights of the participant to refuse to participate in the study without giving a reason must be respected. After the participant has entered into the study, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. The reason for doing so, however, should be recorded; the participant will remain within the study for the purpose of follow-up and for data analysis by the treatment option to which they have been allocated. Similarly, the participant must remain free to change their mind at any time about the protocol treatment and study follow-up without giving a reason and without prejudicing his/her further treatment.

11.3 COMPETENT AUTHORITY APPROVALS

This protocol will be submitted to the national competent authority (MHRA). This is a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is required in the UK. The EudraCT number for the study is 2014-001425-32.

The progress of the study and safety issues will be reported to the competent authority in accordance with local requirements and practices in a timely manner. Safety reports, including expedited reporting and SUSARs will be submitted to the competent authority in accordance with their requirements in a timely manner.

11.4 OTHER APPROVALS

The protocol will be submitted by those delegated to do so to the relevant R&D department of each participating site or to other local departments for approval as required. A copy of the local R&D approval (or other relevant approval as above) and of the Participant Information Leaflet (PIL) and Informed Consent Form (ICF) on local headed paper must be forwarded to the RIVER Co-ordinating Centre before the site can be opened.

12 INDEMNITY

The sponsor of the study, Imperial College, holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this study.

If it can be demonstrated that a participant experienced serious and enduring harm as a result of their participation in this study, they may be eligible to claim compensation without having to prove that Imperial College is at fault. If the injury resulted from any procedure which is not part of the study, Imperial College will not be required to compensate them in this way. Their legal rights to claim compensation for injury where it can be proven as negligence are not affected.

13 FINANCE

The study is supported by grant funding from the Medical Research Council (Ref: MR/L00528X/1).

The study will be co-ordinated by the MRC CTU. A written agreement with the NHS Trust or Board of each site principal investigator (PI) and the MRC CTU will set out the obligations of the parties to the agreement, their respective roles and responsibilities and cover arrangements for budgets and financial transfers and reporting. The study will also be registered through the NIHR portfolio system for adoption by the Comprehensive Local Research Network (CLRN).

Raltegravir and vorinostat - Are manufactured by Merck Sharp and Dohme (MSD). MSD have agreed to supply the study drugs free of charge for participants participating in the study.

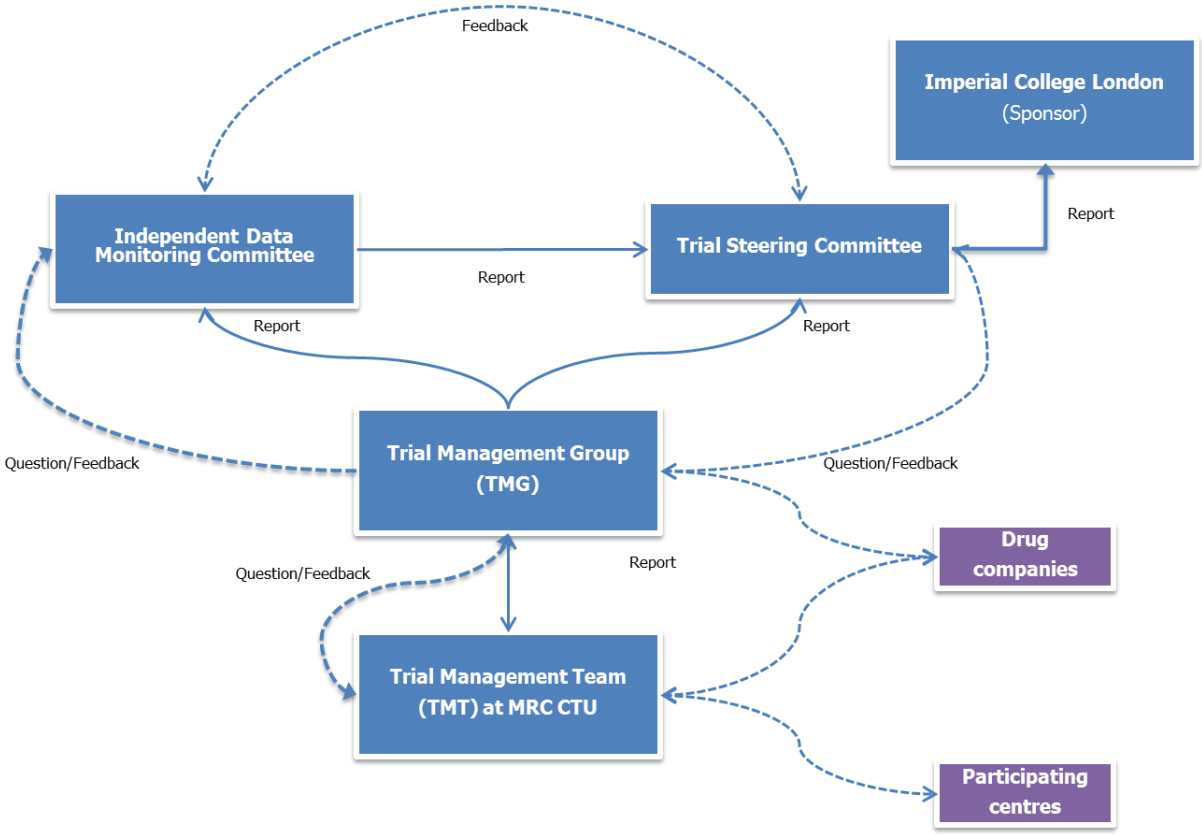
ChAdV63.HIVconsv and MVA.HIVconsv Vaccines - The vaccine manufacture and all pre-clinical and clinical work to date for these vaccines has been provided free of charge to the RIVER study by Lucy Dorrell and Tom Hanke utilising their existing MRC grants. GSK own the ChAd vector and have reviewed the RIVER protocol and have approved the use of the ChAdV63.HIVconsv. in the RIVER study.

Participants will receive reimbursement for their commitment to the study.

14 OVERSIGHT & TRIAL COMMITTEES

There are a number of committees involved with the oversight of the study. These committees are detailed below, and the relationship between them expressed in the **Figure 5** below.

Figure 5 Trial Organogram



14.1 TRIAL MANAGEMENT TEAM (TMT)

A Trial Management Team (TMT) will be formed to conduct the day-to-day management of the study at the MRC CTU. This will include the Chief Investigator, Trial Statistician, Trial Physician, Clinical Project Manager (MRC CTU), Trial Manager and Data Manager. The group will meet at least once per month, although may meet more or less often as required. The group will discuss issues related to the progress of the study at the site and ensure that the study is running well.

14.2 TRIAL MANAGEMENT GROUP (TMG)

A Trial Management Group (TMG) will be formed, comprising of the Chief Investigator, other lead investigators (clinical and non-clinical), members of the MRC CTU (RIVER Co-ordinating Centre) representatives of the sponsor, participant representative and site principal investigators. The TMG will be responsible for the day-to-day running and management of the study. This group will be

chaired by the Chief Investigator and all decisions regarding the overall running of the study will be made in this forum with the exception of matters of fundamental importance to the viability of the study or that require major changes to the protocol. These will be referred to the Trial Steering Committee (TSC). The full details can be found in the TMG Charter. It will meet approximately three times a year, at least one of which will be in-person. At these meetings, progress and challenges will be summarised and difficulties discussed.

14.3 TRIAL STEERING COMMITTEE (TSC)

The Trial Steering Committee (TSC) has membership from the TMG plus independent members, including the Chair. The role of the TSC is to provide overall supervision for the study and provide advice through its independent Chair. The ultimate decision for the continuation of the study lies with the TSC. Further details of TSC functioning are presented in the TSC Charter. See **Appendix V** for membership.

14.4 DATA MONITORING COMMITTEE (DMC)

An Independent Data Monitoring Committee (IDMC) will be formed. The IDMC will be the only group which sees the confidential, accumulating data for the study separately by randomised group. Reports to the IDMC will be produced by the MRC CTU statisticians. The IDMC will review study data on recruitment, safety, adherence to randomised strategies and efficacy, as well as consider findings from any other relevant studies. The frequency of meetings will be dictated in the IDMC charter. The IDMC will consider data using the statistical analysis plan and will advise the TSC. The IDMC can recommend premature closure or reporting of the study, or that recruitment be discontinued.

Further details of IDMC functioning and the procedures for interim analysis and monitoring are provided in the IDMC Charter. **See Appendix V** for membership.

14.5 ROLE OF STUDY SPONSOR

The study will be sponsored by Imperial College London (Imperial) with responsibilities defined by a written agreement

15 PUBLICATION

The results from different centres will be analysed together and published as soon as possible after the end of the study. Individual clinicians must not publish data concerning their participants that are directly relevant to questions posed by the study until the TMG has published its report. The TMG together with the RIVER collaborators will form the basis of the writing committee and decide on the nature of publications. Any release, of efficacy or safety data, presentation or publication will be agreed with the TSC according to the terms of their charter.

All publications will acknowledge the participating centres and clinicians, and these will be detailed in an appendix to the main report. Papers will have named authors determined by the TMG according to the following principles:

- To be as inclusive as possible where this is practicable
- To ensure that there is justification for anyone to be named as an author
- Reasons for nomination for authorship may include: study design; grant holding; day-to-day study oversight (TMG membership); analysis; discussion and interpretation of data; representation for key groups; active participation at large recruiting sites. It should be accepted that the people qualifying for authorship will vary over time. In addition, key positions will vary depending on the nature of the publication: clinical lead for clinical papers, statistician lead for methodology papers, translational papers may be led by authors not on the main TMG if appropriate. In the event of any dispute related to authorship or data release, the TSC will be responsible for making the executive decision.

In the manuscript, a full list of sites and the number of participants recruited will be provided. In the presentations, this list of sites will also be shown. The term “the RIVER investigators” will clearly be stated and relevant names included in the presentation credits.

16 PROTOCOL AMENDMENTS

Protocol v1.0 24-Oct-2014 approved by REC 23-Dec-2014 and MHRA 13-Jan-2015	
Protocol v2.0 03-Jun-2015 approved by REC 25-Jun-2015. Not accepted by MHRA.	
Changes made	**Sections updated**
Schedule amended to allow for repeat testing of a participant's viral load, until this falls below the level of detectability. Trial assessment schedule has been split into two stages, pre and post-randomisation, with randomisation becoming a separate visit to ensure all participants have a common baseline. All visits after randomisation are now referred to as post-randomisation weeks (PR week), which has been updated throughout.	Table 1 – Trial Assessment Schedule Table 2 – Visit names and codes Figure 1 Trial Entry, Randomisation and Treatment 3.2 Criteria for Randomisation 3.3 Number of participants 4. Screening, Enrolment & Randomisation
Eligibility assessments including routine blood results and resting 12-lead ECG need to be within 14 days of randomisation.	Table 1 – Trial Assessment Schedule 3.2 Criteria for Randomisation
Wording clarified for enrolment exclusion criteria 22.	3.1.2 Enrolment Exclusion Criteria
Removed the requirement for participants to attend visits fasted.	Table 1 – Trial Assessment Schedule 6.1.1.A Screening and Enrolment (Week 0)
Changes to the trial assessment schedule only: - Screening/baseline visit is now referred to as screening only. - Follow up visit at week 08 has been removed - For several visits the volumes and timings of blood samples have been changed. Total blood volume taken across the protocol has increased by 17ml. - Visits where quality of life questionnaires are required have changed.	Table 1 – Trial Assessment Schedule
Further clarification on the vaccination visits has been included: - Specified that the first vaccination (Chad) at PR week 00 must take place within 1 week of randomisation. For participants is Arm A, this visit can be completed over the phone. - There is a window of 7 days between the two vaccinations given at PR week 00 and PR08 Day 1.	Table 1 – Trial Assessment Schedule 5.3 Post-Randomisation (PR) Treatment 5.5.3 Vaccines
- Preliminary results from the BCN01 study are now available. - Results from the START study are now available	1.2.5.C Use of ChAdV63.HIVconsv and MVA.HIVconsv in HIV Infection

	11.2.1Ethical Considerations
Event reporting section has been updated : - Only grade 3 adverse events are reportable prior to randomisation. Grade 4 events are reportable throughout the study as SAEs. - Pregnancy in a partner is now considered a notable event - Vaccine related events have been added to the notable events and are listed in Appendix IV. - For SAEs, participants must be identified by study number, 3 letter code and year of birth only. - Clarification that HERVs may be investigated as an exploratory outcome.	7.3 Other Notable Events 7.3.1 Vaccine related events 7.4 Investigator Responsibilities 7.4.2Notification Procedure
- Re-clarification of the end of the interventional phase of the study and end of study.	7.4.1.E Notification 11.1 Compliance
Change in staff at the RIVER Co-ordinating centre and PI at Brighton and Sussex University Hospitals NHS Trust. Protocol version number and date updated on first page and on all page headers.	
Protocol v3.0 23-Jul-2015	
Changes made	**Sections updated**
Re-clarification of the investigator's event reporting responsibilities.	7.4.1.E Notification

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