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NCT Number	NCT03371485
EudraCT Number	2016-002577-35
Sponsor Protocol Number	CRUKD/17/003
Official Title	A Cancer Research UK Phase I trial of AST-VAC2 (allogeneic dendritic cell vaccine) administered weekly via intradermal injection in patients with advanced non-small cell lung cancer.
Document, Version & Date	Protocol Version 5.0, dated 08Jun2021

CANCER RESEARCH UK

Centre for Drug Development

A Cancer Research UK Phase I trial of AST-VAC2 (allogeneic dendritic cell vaccine) administered weekly via intradermal injection in patients with advanced non-small cell lung cancer.

Sponsor protocol number: CRUKD/17/003

EudraCT number: 2016-002577-35

Chief Investigator: Professor Christian Ottensmeier

[redacted]

Sponsor: Cancer Research UK

[redacted]

PARTICIPATING INVESTIGATORS AND CENTRES:

Details of Principal Investigators and Investigational Sites are recorded on the Participating Investigators and Centres list in the Sponsor's Trial Master File.

VERSION HISTORY:

Version No.	Date of issue	Reason for update
1.0	03MAR2017	Initial version for REC/HRA and MHRA submission.
2.0	15MAY2017	Revised version post initial review by the MHRA and REC.
3.0	18JUL2017	Revised version post 2nd review by the REC.
4.0	19NOV2018	Protocol Amendment 01: Clarification that the requirement for dose delays excludes AEs of ISR, amendment to the exclusion criteria regarding the use of prohibited concomitant steroid medication, amendment to when the expansion cohort can open, requirement for related AEs meeting the stopping rule criteria to be reported as an SAE added, caveat around the use of systemic steroids to treat AEs added, change in container closure system used for the IMP, clarification on imaging requirements and objectives, [redacted] change to the lymphocyte count required for inclusion into the study. Other minor changes and clarifications.
5.0	08JUN2021	Protocol Amendment 02: Removal of the control arm in the safety cohort and removal of the expansion cohort; change to end of trial definition; changes to reasons for withdrawal; other minor changes and clarifications.

TABLE OF CONTENTS

[redacted]

[redacted]

[redacted]

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

	Abbreviation	Definition
A	ABPI	Association of the British Pharmaceutical Industry
	AE	adverse event
	ALK	anaplastic lymphoma kinase
	ALP	alkaline phosphatase
	ALT	alanine aminotransferase
	AML	acute myeloid lymphoma
	ANA	anti-nuclear antibodies
	ANC	absolute neutrophil count
	AST	aspartate aminotransferase
	ATIMP	advanced therapy investigational medicinal product
B	BDU	Biotherapeutics Development Unit
	BP	blood pressure
	BUN	blood urea nitrogen
C	CDD	Centre for Drug Development
	CEA	carcinoembryonic antigen
	CI	Chief Investigator
	CR	complete response
	CRA	Clinical Research Associate
	CRP	c-reactive protein
	CRUK	Cancer Research UK
	CSM	Clinical Study Manager
	CSR	clinical study report
	CT	computerised tomography
	CTA	Clinical Trial Authorisation
	CTCAE	Common Terminology Criteria for Adverse Events
	CXR	chest x-ray
D	DC	dendritic cell
	DFS	disease free survival
	DMSO	dimethylsulfoxide
E	ECG	electrocardiogram
	eCRF	electronic case report form
	EDC	electronic data capture
	[redacted]	[redacted]
	ELISPOT	enzyme-linked immunospot
	EoT	End of Trial
	EP	early progression
	ESMO	European Society for Medical Oncology
[redacted]	[redacted]	[redacted]
G	GCP	Good Clinical Practice
	GLP	Good Laboratory Practice
	GMP	Good Manufacturing Practice
	GP	general practitioner
H	Hb	haemoglobin
	HCG	human chorionic gonadotropin
	hESC	human embryonic stem cells
	HIV	human immunodeficiency virus
	HLA	human leucocyte antigen
	HRA	Health Research Authority
	hTERT	human telomerase reverse transcriptase
I	IB	investigator's brochure
	ICD	informed consent document
	ICH GCP	International Conference on Harmonisation of Good Clinical Practice

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

	Abbreviation	Definition
	id	intradermal
	[redacted]	[redacted]
	IMP	investigational medicinal product
	IND	investigational new drug
	irCR	immune-related complete response
	irPR	immune-related partial response
	irPD	immune-related progressive disease
	irRC	immune-related response criteria
	irSD	immune-related stable disease
	ISR	injection site reaction
	ITF	Investigator Trial File
	IUD	intrauterine device
L	[redacted]	[redacted]
M	MIA	manufacturing authorisation holder
	[redacted]	[redacted]
	MHRA	Medicines and Healthcare products Regulatory Agency
	[redacted]	[redacted]
	MRI	magnetic resonance imaging
	mRNA	messenger ribonucleic acid
N	NCI	National Cancer Institute
	NGS	Next Generation Sequencing
	NHS	National Health Service
	NK	natural killer
	NSCLC	non-small cell lung cancer
	NYHA	New York Heart Association
O	OS	overall survival
P	PBM	peripheral blood monocytes
	PCR	polymerase chain reaction
	PD	progressive disease
	PDF	portable document format
	pH	power of hydrogen (concentration of the hydrogen ion)
	PI	Principal Investigator
	PoP	proof of principle
	PR	partial response
	PS	performance status
	PSRB	Protocol and Safety Review Board
Q	QA	quality assurance
	QC	quality control
	QP	Qualified Person
R	REC	Research Ethics Committee **(for the purposes of this trial, the Gene Therapy Advisory Committee will serve as the REC).**
	RECIST	Response Evaluation Criteria in Solid Tumours
	RF	rheumatoid factor
	RFS	relapse free survival
	RNA	ribonucleic acid
	[redacted]	[redacted]
S	SAE	serious adverse event
	SD	stable disease

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

	Abbreviation	Definition
	SDV	source data verification
	SOP	standard operating procedure
	SPD	sum of products of the two largest perpendicular diameters
	SUSAR	suspected unexpected serious adverse (drug) reaction
T	TMF	Trial Master File
	TSH	thyroid stimulating hormone
U	[redacted]	[redacted]
	UK	United Kingdom
	ULN	upper limit of normal
	USM	urgent safety measure
W	WBC	white blood cell
	WHO	World Health Organisation

PROTOCOL SIGNATURES

Sponsor Signature:

The Sponsor has read and agrees to the protocol, as detailed in this document. I am aware of my responsibilities as the Sponsor under the UK Clinical Trials Regulations[1], the guidelines of Good Clinical Practice (GCP)[2], the Declaration of Helsinki[3], the applicable regulations of UK law and the trial protocol. The Sponsor agrees to conduct the trial according to these regulations and guidelines and to appropriately direct and assist Sponsor's staff who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.

Name: _____

Title: _____

Signature: _____

Date: _____

1 The Medicines for Human Use (Clinical Trials) Regulations (S.I. 2004/1031) and any subsequent amendments to it.

2 ICH Harmonised Tripartite Guideline E6 (R1): Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) Step 5, adopted by CPMP July 1996.

3 WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and all subsequent amendments including Oct 2013.

PROTOCOL SIGNATURES

Investigator Signature:

I have read and agree to the protocol, as detailed in this document. I am aware of my responsibilities as an Investigator under the UK Clinical Trials Regulations[1], the guidelines of Good Clinical Practice (GCP)[2], the Declaration of Helsinki[3], the applicable regulations of the relevant NHS Trusts and the trial protocol. I agree to conduct the trial according to these regulations and guidelines and to appropriately direct and assist the staff under my control, who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.

Investigator's Name: _____

Name of site: _____

Signature: _____

Date: _____

1 The Medicines for Human Use (Clinical Trials) Regulations (S.I. 2004/1031) and any subsequent amendments to it.

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3 WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and all subsequent amendments including Oct 2013.

1 PROTOCOL SYNOPSIS

1.1 Full title:

A Cancer Research UK Phase I trial of AST-VAC2 (allogeneic dendritic cell vaccine) administered weekly via intradermal injection in patients with advanced non-small cell lung cancer (NSCLC).

1.2 Short title:

A Phase I trial of AST-VAC2 vaccine in patients with non-small cell lung cancer.

1.3 Clinical trial objectives and endpoints:

Primary objective	Endpoint
To assess the safety and tolerability of the target dose of AST-VAC2 given as six intradermal (id) injections at Weeks 1, 2, 3, 4, 5 and 6 in patients with advanced NSCLC.	Determining frequency and causality of each adverse event (AE) to AST-VAC2 and grading severity according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.02 or protocol specific grading system for injection site reactions (ISRs).

The secondary and tertiary objectives and endpoints of the trial can be found in Section 3.1.

1.4 Trial Design:

This is a first in man, open label, multi-centre Phase I trial to investigate the safety, immunogenicity and patient survival following administration of the dendritic cell (DC) vaccine AST-VAC2 in patients with advanced NSCLC (metastatic or locally advanced).

All enrolled patients will receive AST-VAC2.

Safety review and stopping rule criteria are detailed in Section 3.3. If stopping criteria are met as per protocol, this will trigger an independent review of the data. Pharmacodynamic data will also be reviewed as available.

All potential trial patients will be asked to consent to a pre-screening assessment of Human Leucocyte Antigen (HLA) typing. Once the HLA result is known, patients who are HLA A*02:01 positive will be asked to consent to participate in the main trial. Patients who are HLA A*02:01 negative will not be eligible for the trial.

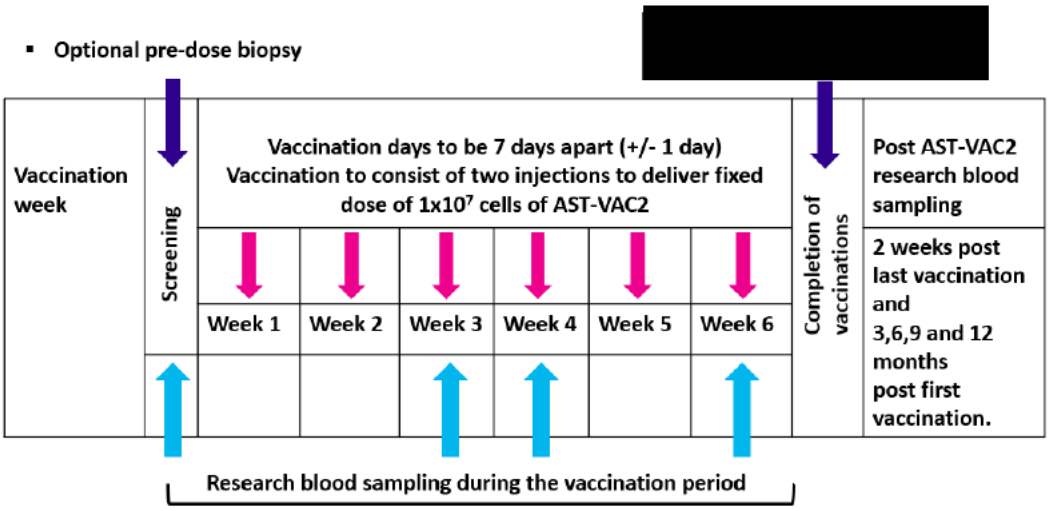
Patients receiving AST-VAC2 will be followed up for safety (primary endpoint), pharmacodynamic analyses in blood samples (secondary endpoint) [redacted].

All patients will be followed up for up to two years post first vaccination for overall survival and for five years post first vaccination for safety.

1.5 Trial treatment:

Patients will receive AST-VAC2 vaccinations, administered by id injection at six time points over a period of six weeks (Figure 1). An interval of 7 days (+/- 1 day) should occur between each of the vaccination days. Two intradermal injections (totalling $1x10^7$ cells of AST-VAC2) will be administered on each vaccination day. The target administered dose of vaccine is $1x10^7$ cells of AST-VAC2.

Figure 1. Summary of vaccinations and research sampling



* Timings may change if vaccinations are stopped before the sixth vaccination

1.6 Patient Population:

Up to eight patients with advanced NSCLC will be recruited to the trial. The final number will depend on the number of patient replacements required and vaccine availability.

2 INTRODUCTION

2.1 Background

2.1.1 Background on non-small cell lung cancer

Primary lung cancer is the most common malignancy after non-melanocytic skin cancer with deaths from lung cancer exceeding those from any other type of malignancy worldwide. While it has been the most important cause of cancer mortality in men since the 1960s, it has equalled breast cancer as a cause of mortality in women since the 1990s. Lung cancer is still increasing both in incidence and mortality worldwide. Non-small-cell lung cancers (NSCLC) account for 85%-90% of lung cancers [1].In 2014 there were 46,403 new cases of lung cancer in the United Kingdom (UK) [2].

The current treatment strategy for lung cancer should take into account histology, molecular pathology, age, Performance Status (PS), comorbidities, and patient's preference. The European Society for Medical Oncology (ESMO) clinical practice guidelines [3] recommend for the treatment of early stages I and II NSCLC, surgery should be offered to patients who are willing to accept procedure-related risks. The ESMO clinical practice guidelines recommend the first line treatment strategy for locally advanced stage III or metastatic NSCLC patients is a platinum –based doublet chemotherapy, cisplatin-based regimens delivered concurrently with radiotherapy have been studied most extensively and are therefore recommended [1], [3]. Cisplatin based regimens (e.g. cisplatin-etoposide or cisplatin-vinorelbine) have shown higher response rates when compared with carboplatin combinations. The overall survival (OS) was significantly superior for cisplatin in the subgroup of non-squamous cell tumours and in patients treated with third-generation regimens, including gemcitabine and taxanes in one meta–analysis. However, cisplatin-based chemotherapy is associated with more digestive, neuro-, and nephrotoxicity, while hematotoxicity is more often observed with carboplatin [1].

Patients clinically or radiologically progressing after first-line chemotherapy with PS 0-2 should be offered second-line chemotherapy. Comparable options as the second-line therapy consist of pemetrexed or docetaxel. In accordance with ESMO clinical practice guidelines, pemetrexed is preferred to gemcitabine or docetaxel in patients with non-squamous tumours. Pemetrexed use should be restricted to non-squamous NSCLC in any line of treatment [1].

There is increasing interest in identifying sub-populations with potentially treatable molecular abnormalities. Targeted therapies such as tyrosine kinase inhibitors (erlotinib, gefitinib) play a role in patients who have an activating mutation in the relevant pathways such as the epidermal growth factor receptor. Translocations in c-met/anaplastic lymphoma kinase (ALK) or proto-oncogene 1, receptor tyrosine kinase (ROS) can be targeted by crizotinib, another tyrosine kinase inhibitor; however only a minority (<20%) of patients benefit.

Approximately half (47%) of patients diagnosed with lung cancer present with Stage IV disease [2], a fact that is reflected in the survival rates: 32% (1 year), 10% (5 year) and 5% (10 year) [2].

It is estimated that patients with advanced disease, who would be eligible for inclusion in this trial, account for about 47% (Stage IV advanced disease at diagnosis) [2] of the overall lung cancer population.

2.2 Investigational medicinal product

For additional information concerning AST-VAC2, refer to the Investigator Brochure (IB).

2.2.1 Structure of AST-VAC2

[redacted]

[redacted]

[redacted]

[redacted]

2.2.2 Mechanism of action of AST-VAC2

AST-VAC2 is a DC cancer vaccine which induces an immunogenic response against the tumour associated antigen hTERT.

2.3 Safety considerations for the trial

2.3.1 Non-clinical pharmacology

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.3.2 Pharmacokinetics

[redacted]

2.3.3 Toxicology

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.4 Clinical experience (other compounds in the same class)

This protocol represents a first-in-human clinical trial, accordingly there is no clinical trial experience with AST-VAC2 to date. However there are clinical data generated form Phase I and II oncology trials which administered an investigational agent analogous to AST-VAC2, the autologous DC therapy AST-VAC1. [redacted]

The early phase, proof-of-concept trials supporting AST-VAC1 development were conducted at Duke University Medical Center under investigator-sponsored investigational new drug applications (INDs). These trials enrolled 51 patients and treated 43 patients between December 2001 and May 2006. The majority of trial subjects had metastatic prostate cancer, and lesser numbers had renal cell carcinoma, chronic myeloid leukaemia or chronic lymphocytic leukaemia.

For comprehensive previous clinical data for the above trials, please see the current Investigator Brochure, Section 5.1.2.1.

2.5 Rationale for the trial

Novel immunotherapy agents, such as anti-CTLA4 and anti-PD-1 antibodies, as well as vaccines such as TG4010, have demonstrated that NSCLC is amenable to immunotherapy [26-28, 30].

hTERT may be a valuable target in NSCLC. hTERT-specific immune responses were seen in 29 of 44 patients following hTERT peptide (amino acids 611-626 and 540-548) vaccination in patients with Stage III NSCLC, and with immune responders achieving increased survival compared to non-responders. Two subjects are free of disease 8-9 years post-vaccination [13, 14].

While these agents appear to improve outcome, they remain a long way from being able to offer durable term disease control to more than a fraction of patients [29]. The promise that cancer immunotherapy may offer along with the novel properties of AST-VAC2 suggest that the proposed trial is a worthwhile undertaking.

AST-VAC2 has a defined HLA Class I and Class II profile. [redacted] The HLA A*02:01 locus is expressed at high frequencies in the general population, including in approximately 46% of individuals of North European Caucasian ancestry [4, 5]. In order to ensure that the patients enrolled in this trial are allogeneically compatible with the hTERT/DC system and thus able to benefit from any therapeutic potential offered, they will be phenotyped prior to inclusion to ensure that they are also HLA A*02:01 positive. No attempt will be made to match patients to the remaining alleles as it is hoped that this mismatch will induce a beneficial mixed leukocyte reaction.

2.5.1 Justification for dose and schedule

The dose of AST-VAC2 proposed for use in the current trial has been selected based on previous clinical experience with autologous DCs [redacted] in which a dose of $1x10^7$ cells was shown to induce anti-hTERT T cell responses in patients with either prostate cancer or acute

myelogenous leukaemia (see Section 2.4 and IB Section 5.1.2.1). In this trial, a target dose of $1x10^7$ cells has been proposed based on the tolerability of this dose in the AST-VAC1 trial discussed in Section 2.4 and IB Section 5.1.2.1.

Each patient will receive a maximum of six vaccinations based on previous clinical experience with AST-VAC1 in prostate cancer patients, where six vaccinations were found to induce the highest levels of anti-hTERT immune responses. Based on the vaccination regimens used for AST-VAC1, AST-VAC2 will be given at Weeks 1, 2, 3, 4, 5 and 6, 7 days apart (+/- 1 day).

3 TRIAL DESIGN

3.1 Clinical trial objectives and endpoints

3.1.1 Primary objectives and endpoints

Primary objective	Endpoint
To assess the safety and tolerability of the target dose of AST-VAC2 given as six id injections at Weeks 1, 2, 3, 4, 5 and 6 in patients with advanced NSCLC.	Determining the frequency and causality of each adverse event to AST-VAC2 and grading severity according to the NCI CTCAE Version 4.02 or protocol specific grading system for ISRs.

3.1.2 Secondary objectives and endpoints

Secondary objectives	Endpoint
To determine the immunogenicity (peripheral response) of AST-VAC2 in patients with advanced NSCLC.	Observation of the total number of patients showing durable peripheral immune response, defined as a change in one validated assay at two time points after at least two vaccinations. Immunological response will be measured in patient blood by hTERT specific T cells as measured by ELISPOT analysis at the following time points: • baseline, • vaccination weeks 3, 4 and 6, • 2 weeks post last vaccination and • 3, 6 and 12 months post first vaccination. Immunological change after vaccination is defined as 2.5 fold change over baseline (ratio baseline to post-treatment samples). Individual patient timepoints will be reported as SFU/10^6 PBMCs. Sample will be considered reportable when spot count of peptide pool is greater than peptide pool specific cut-off (as defined in validation).
To document any tumour response(s) in patients receiving the AST-VAC2 vaccine.	Re-assessment of tumour size at end of vaccination visit with Magnetic Resonance Imaging (MRI) or Computerised Tomography (CT) using Immune-Related Response Criteria (irRC).
To determine the 2-year overall survival (OS) in patients receiving the AST-VAC2 vaccine.	Measurement of overall survival at two years post first vaccination.

3.1.3 Tertiary objectives and endpoints

Tertiary objective	Endpoint
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

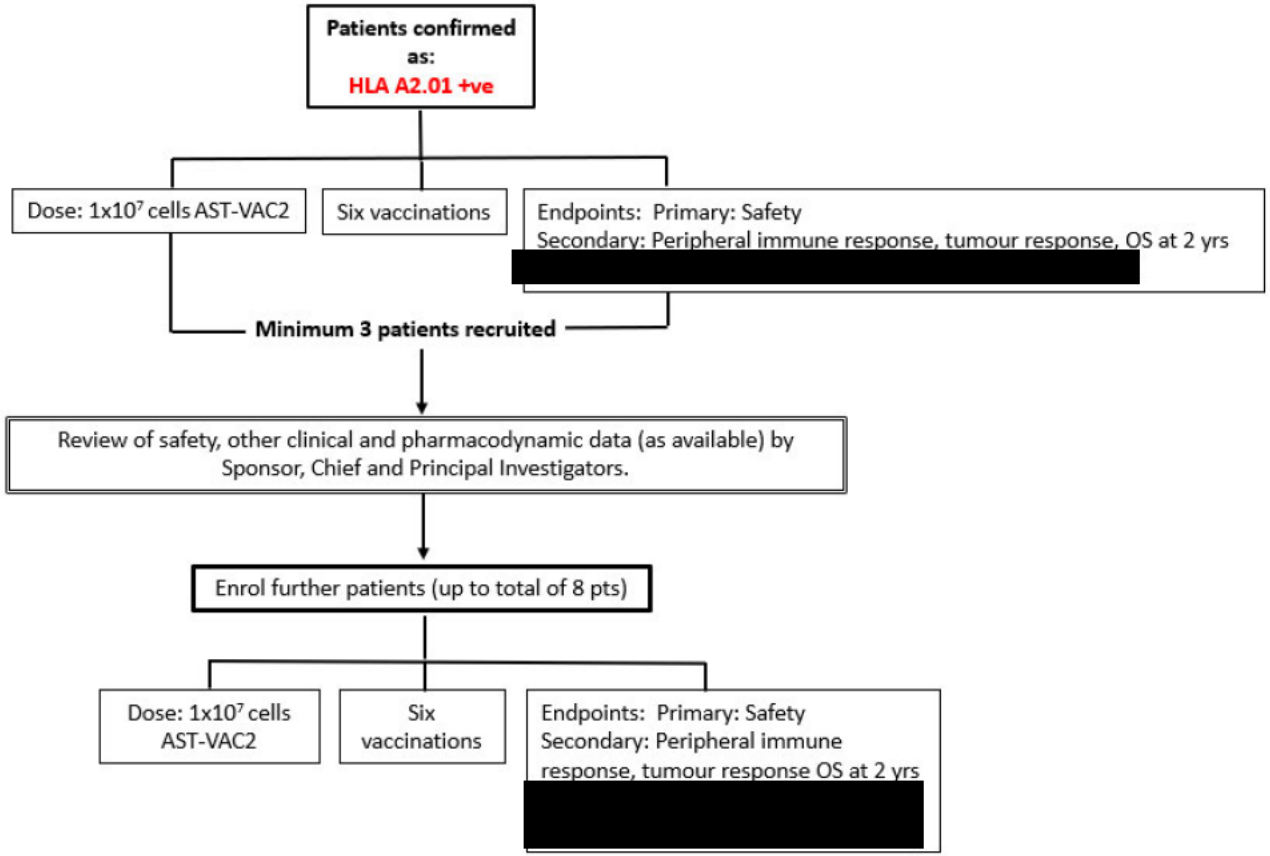
3.2 Design of the clinical trial

This is a first in man, open label, multi-centre Phase I trial to investigate the safety, immunogenicity and patient survival when administered the DC vaccine, AST-VAC2, in patients with advanced NSCLC (metastatic or locally advanced disease).

All enrolled patients will receive AST-VAC2 to establish if the vaccine can be delivered with an acceptable safety profile and to investigate immune response in peripheral blood, [redacted] See Figure 2.

Safety review and stopping rule criteria are detailed in Section 3.3. The study data will undergo independent review if stopping rule criteria are met. Staggered patient recruitment will be employed in order for emerging safety data to be reviewed before further patients are recruited (Section 3.3.2).

Figure 2. Overall Trial design



All potential patients will be asked to consent to a pre-screening assessment of HLA typing. Only patients who are screened and found to be HLA A*02:01 positive will be asked to consent to the main trial and continue into screening to establish eligibility for trial treatment.

It is expected that up to eight patients will be recruited, the final number depending on the emerging safety data, patient replacement and vaccine availability.

Patients will receive six AST-VAC2 vaccinations (administered by two id injections for each vaccination), once a week (every 7 days +/- 1 day) for six weeks. Only one target dose of AST-VAC2 will be explored i.e. $1x10^7$ cells. A reduced dose of AST-VAC2 or a reduction in number of vaccinations administered, may be considered following observation of unacceptable toxicity at this dose level. Any modification to the vaccination dose/schedule will be determined and agreed by the Sponsor & Chief Investigator based on emerging data as the trial progresses.

The 'End of Trial' will be declared once all patients have either withdrawn (see Section 11.0) from the trial or died, or the last patient has completed their final safety follow-up visit (5 years post the first vaccination), whichever is the latter.

3.3 Safety Review

3.3.1 Safety considerations

AST-VAC2 has never been investigated in the clinic so no clinical safety data are available. Based upon experience with other autologous and allogeneic DC vaccines, including AST-VAC1, the potential toxicities of AST-VAC2 are expected to be minimal. Severe AEs related to AST-VAC1 (related autologous DC cell therapy, Section 2.4 and IB section 5.1.2.1) have not been observed in Phase I and II clinical trials with the possible exception of an occurrence of thrombocytopenia in one patient with acute myeloid leukaemia (AML).

The principal potential toxicity of AST-VAC2 is likely to be related to the allogeneic nature of the AST-VAC2 vaccine which could lead to injection site inflammation. However, it is noted that the mixed leucocyte reaction could elicit an adjuvant effect which may be beneficial in the context of this trial. Systemic inflammatory responses associated with cytokine release have not been demonstrated in dendritic cell vaccines to date and therefore are not considered to be a significant risk for AST-VAC2.

Chronic toxicities, particularly related to unintended proliferation of AST-VAC2, are not anticipated. AST-VAC2 is derived from a hESC line. The cells are terminally differentiated during manufacture to generate cells with a phenotype comparable to that of autologous dendritic cells, which are known to have a low proliferative potential and a short biological half-life. Furthermore, these cells are irradiated post-cryopreservation to minimise the risk of any cells with proliferative potential remaining in the IMP. Finally, AST-VAC2 is only partially haplotyped to the recipient therefore the recipient may also induce a rejection reaction to the cells causing rapid elimination. Overall, the drug product is therefore expected to have no proliferative potential in vivo and the potential for tumourgenicity/ectopic growth is considered to be remote.

All AEs observed during this trial will be assessed using NCI CTCAE Version 4.02, with the exception of Injection Site Reactions (ISR) which are defined using the protocol specific grading system detailed in Section 9.8.

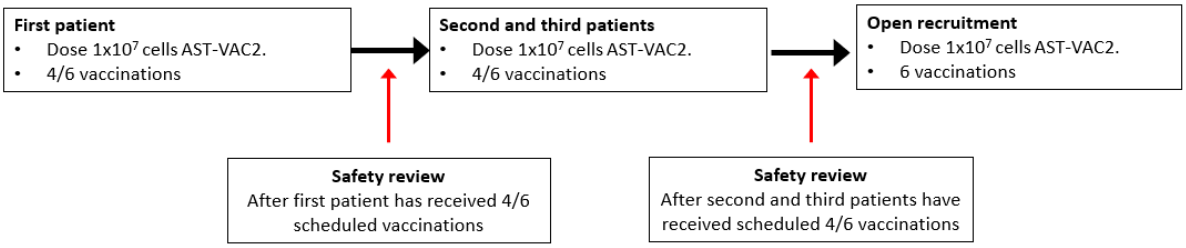
The risk of developing any of the AEs noted above and in Section 3.3.3 is low and largely theoretical however if any of the above is seen or suspected during the vaccination schedule or follow up period, investigators should investigate as per standard clinical practice and may include assessments as appropriate. If any of these AEs meet the stopping rule criteria as set out in Section 3.3.3, this will trigger an independent review as stated.

3.3.2 Safety Evaluation

Only one target dose level is being explored in this trial.

Safety evaluations by the Sponsor and collaborating Investigators will be conducted after the first patient has received AST-VAC2 and then again after the second and third patients have been treated, Figure 4. Once open recruitment has been initiated, safety review will be performed at regular intervals (as required).

Figure 3. Safety Evaluation



A safety review committee, consisting of all Investigators, representatives of the Sponsor and representatives of the drug company (observers only), will review emerging study data (to include all clinical data, safety data listings and applicable PD data) on an ongoing basis, with particular reference to ensuring that patients are able to complete the vaccination schedule without AST-VAC2 toxicity-related delays. Should the committee have significant concerns about toxicity seen during the trial they will have the authority to suspend or terminate the trial and/or make recommendations to the Sponsor to modify the vaccination administration.

Pending discussions between the Sponsor and CI and based on vaccine availability, the following options may be considered during the trial:

- Reduced dose and/or
- Modified number of vaccinations, e.g. if plateaued immune responses are observed with fewer vaccinations in those patients already treated.

3.3.2.1 Data to be discussed at safety review meetings.

Data which should be discussed at the safety review meeting will include:

- Patients treated since the last safety review meeting including vaccine related adverse events noted and duration of treatment.
- Assessment and agreement on any related adverse events that may have occurred and resulting actions.
- Relevant PD data available since the last meeting.
- Any additional relevant information relating to adverse events or patient safety which may have arisen following distribution of listings for the meeting and will therefore not be documented in the listings.
- Any possible concern after the review of cumulative data e.g. toxicities.
- Assessment and agreement on continuation of patient enrolment to the study.

3.3.3 Stopping rule criteria.

On observation of any of the following related adverse events, the study data (defined above) will be reviewed by the independent Protocol Safety and Review Board (PSRB) for an opinion.

- ISR Grade 3 or 4 (as per protocol specific grading system).
- Clinically significant systemic allergic reaction (in the judgement of the investigator)
- Evidence of clinically significant autoimmunity (in the judgement of the investigator)
- Cytokine release syndrome Grade 3 or 4.
- Evidence of tumorgenicity/ectopic growth (including teratoma formation).
- Any other clinically significant toxicity (in the judgement of the investigator)

Any incidences of the related adverse events detailed here must be reported to the Sponsor as a medically important serious adverse event (SAE) (see Section 9.2.2).

3.4 Patient evaluability

3.4.1 Response

All patients who meet the eligibility criteria, receive at least one AST-VAC2 vaccination, have a baseline assessment and at least one repeat assessment of measurable disease will be evaluable for response. Response is not a primary endpoint however patients will be assessed for any potential responses to trial treatment. Disease will be measured according to the modified Immune-related Response Criteria (irRC) (Appendix 3).

3.4.2 Safety

All patients who meet the eligibility criteria and receive at least one administration of AST-VAC2 will be evaluable for the assessment of the primary endpoint of safety.

3.4.3 Immunogenicity

Patients will be defined as "immune evaluable" for the secondary immunogenicity endpoints if the criteria below are met:

- Has received at least three scheduled AST-VAC2 vaccinations (three vaccinations have been shown to be adequate to measure a peripheral immune response) and;

- Has provided a baseline research blood sample plus samples at two subsequent sampling time points. Every effort should be made to collect the sample 2 weeks after the last vaccination (Section 8.0) and/or

[redacted]

If no immune response is seen at the three month time point, later time points may not be analysed. If the patient consents, any remaining samples not yet analysed will be considered a research 'gift' before future research is performed on the sample.

4 PATIENT SELECTION

4.1 Eligibility criteria

The patient must fulfil the eligibility criteria (listed in Sections 4.1.1 and 4.1.2).

4.1.1 Inclusion criteria

1. Patients with advanced NSCLC (metastatic or locally advanced), for whom there are no other suitable treatment options –
   - Able to and likely to be well enough to receive six vaccinations i.e. judged by the Investigator to not require alternate treatment for the duration of the vaccination schedule and period to end of vaccination visit.
   - Has had sufficient wash out periods from previous treatments as follows:
     i) four weeks for chemotherapy
     ii) six weeks for investigational medicinal products (IMPs)
     iiii) eight weeks for immunotherapy (shorter intervals may be acceptable based on half-life of treatment. Eligibility will be confirmed by the Sponsor and CI).
   - Measurable disease
   - Biopsiable disease is preferable however patients without biopsiable disease can still be considered for the study.
2. Written (signed and dated) informed consent and be capable of co-operating with treatment and follow-up.
3. Confirmed HLA A*02:01 positive genotype.
4. Life expectancy of at least 12 weeks.
5. World Health Organisation (WHO) performance status of 0-2.
6. Haematological and biochemical indices within the ranges shown below. These measurements must be performed prior to the patient receiving the first AST-VAC2 vaccination.

Laboratory Test	Value required
Haemoglobin (Hb)	≥9.0 g/dL
Absolute neutrophil count (ANC)	≥1.5 x 10^9/L
Platelet count	≥100 x 10^9/L
Lymphocyte count	≥1.0 x 10^9/L
Bilirubin	≤1.5 x upper limit of normal (ULN)
Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) and alkaline phosphatase (ALP)	≤ 3.0 x ULN
Calculated creatinine clearance	> 30 mL/min

7. 18 years or over at the time consent is given.

4.1.2 Exclusion criteria

1. Radiotherapy (except for palliative reasons) during the previous four weeks before treatment.
2. Ongoing toxic manifestations of previous treatments greater than CTCAE Grade 1. Exceptions to this are alopecia or certain Grade 2 toxicities, which in the opinion of the Investigator should not exclude the patient. If there is uncertainty over whether the toxicity should exclude the patient, the Sponsor should be consulted.

3. Systemic steroids or other drugs with a likely effect on immune competence are forbidden during the trial with the exception of replacement treatment. Inhaled and topical steroids are permitted. The predictable need of their use will preclude the patient from trial entry.

4. Female patients who are able to become pregnant (or are already pregnant or lactating). However, those patients who have a negative serum or urine pregnancy test before enrolment and agree to use two forms of contraception (one effective form plus a barrier method) [oral, injected or implanted hormonal contraception and condom; intra-uterine device and condom; diaphragm with spermicidal gel and condom] or agree to sexual abstinence*, effective from the first administration of AST-VAC2 throughout the trial and for six months afterwards are considered eligible.

5. Male patients with partners of child-bearing potential (unless they agree to take measures not to father children by using a barrier method of contraception [condom plus spermicide] or to sexual abstinence* effective from the first administration of AST-VAC2, throughout the trial and for six months afterwards. Men with partners of child-bearing potential must also be willing to ensure that their partner uses an effective method of contraception for the same duration for example, hormonal contraception, intrauterine device, diaphragm with spermicidal gel or sexual abstinence). Men with pregnant or lactating partners must be advised to use barrier method contraception (for example, condom plus spermicidal gel) to prevent exposure of the foetus or neonate.

6. Major thoracic or abdominal surgery from which the patient has not yet recovered.

7. At high medical risk because of non-malignant systemic disease including active uncontrolled infection.

8. Known to be serologically positive for Hepatitis B, Hepatitis C or Human Immunodeficiency Virus (HIV).

9. Evidence of any ongoing active autoimmune disease.

10. Concurrent congestive heart failure, prior history of class III/ IV cardiac disease (New York Heart Association [NYHA]), prior history of cardiac ischaemia or prior history of cardiac arrhythmia.

11. Is a participant or plans to participate in another interventional clinical trial, whilst taking part in this Phase I trial of AST-VAC2. Participation in an observational trial or interventional clinical trial which does not involve administration of an IMP and which would not place an unacceptable burden on the patient in the opinion of the Investigator and Medical Advisor would be acceptable.

12. Any vaccination given within four weeks before the first AST-VAC2 vaccination (except for COVID-19 vaccinations, which are permitted at the Investigator's discretion).

13. Any planned prophylactic vaccination from trial entry until completion of the AST-VAC2 vaccinations (except for planned COVID-19 vaccinations, which are permitted at the Investigator's discretion).

14. Any condition which might interfere with the patient's ability to generate an immune response.

15. Any other condition which in the Investigator's opinion would not make the patient a good candidate for the clinical trial.

* Abstinence is only considered to be an acceptable method of contraception when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

4.2 Patient enrolment

Before enrolling the patient in the trial, the Investigator or designated representative should determine the eligibility of the patient during the trial screening period. Please ensure that the Sponsor is notified of any eligibility concerns at least four working days before treatment is planned.

Eligible patients must be enrolled in the electronic data capture (EDC) system by site staff and then registered by the Sponsor before they start treatment with AST-VAC2.

Eligible patients will be allocated a trial number by the EDC system during the enrolment process. The Sponsor will send confirmation of the patient registration and dose level, to the investigator following enrolment of the patient; trial treatment may only be administered after this confirmation has been received.

5 TREATMENT

5.1 Selection of the Phase I clinical dose and schedule

The therapeutic vaccine will be administered via two intradermal (id) injections at a target dose of $1x10^7$ cells on each vaccination day. The actual cell dose patients may receive will vary, up to a maximum of $2 x10^7$ viable cells, to achieve a target dose of $1 x 10^7$ viable cells – Section 6.2.5 for further details on dose.

The dose of AST-VAC2 has been selected based on previous clinical experience with autologous dendritic cells (DCs) [redacted] in which a dose of $1x10^7$ cells was shown to induce anti-hTERT T cell responses in patients with either prostate cancer or AML.

5.2 Dosing schedule/treatment schedule

Patients will receive six AST-VAC2 vaccinations, administered by id injection weekly over a period of six weeks. An interval of 7 days (+/-1 day) should occur between each of the vaccination days. The target total administered dose of vaccine is $1x10^7$ cells of AST-VAC2 which will be administered as two id injections for each vaccination.

AST-VAC2 vaccine will be given by id injection in the deltoid muscle of the arm, the outer thigh surface, anterior abdominal wall or buttock. If an injection site reaction (ISR) has resolved after the first vaccination /previous vaccination days then for subsequent injections, injections can be administered at the same body site. If an ISR from a previous vaccination is still present (≥Grade 1) then the injections should be administered into an alternative site.

Only one dose of AST-VAC2 will be explored in this trial. A reduced dose/administration schedule may be considered as per Section 3.3 based on emerging safety data.

5.3 Enrolment

Initially, one patient will be recruited and will receive their first four vaccinations before the second and third patients are recruited. It is acceptable for these next patients to receive treatment in parallel. The second and third patients must receive their first four vaccinations before recruitment is opened to the remaining patients.

Safety evaluations by the Sponsor and collaborating Investigators (Section 3.3) will be conducted after the first patient has received AST-VAC2 and then again after the second and third patients have been treated. Once open recruitment has been initiated, safety review will be performed at regular intervals (as required).

5.3.1 Dose reductions

Pending discussions between the Sponsor and Chief Investigator (CI) and based on vaccine availability, the following options may be considered during the trial:

- Reduced dose and/or
- Modified number of vaccinations, e.g. if plateaued immune responses are observed with fewer vaccinations in those patients already treated.

5.3.2 Dose delays & interruptions

If at the time of the next scheduled vaccination day, one or more adverse events (AEs) (excluding ISRs) related to the AST-VAC2 vaccine (highly probably, probably or possibly related) are still present, have not resolved to Grade ≤1 and are considered by the Investigator to be clinically significant and a clear contraindication to vaccination, the next vaccination day should be delayed for up to two weeks until the AE(s) have recovered to this level. In case of uncertainty, individual cases should be discussed with the Sponsor. Patients who require a dose interruption of greater than two weeks will be reviewed on a case by case basis to determine whether the patient should be withdrawn from the trial. The Sponsor will confirm whether an extended interruption is acceptable or if the patient should be withdrawn from further trial treatment.

If the patient is administered a COVID-19 vaccination during the AST-VAC2 vaccination period, there should be a 48 hours interval between the two vaccinations and they should be administered to different sites. The AST-VAC2 vaccination should be delayed as needed to accommodate this interval.

If the patient is withdrawn from further trial treatment, follow up information and post vaccination research blood [redacted] sampling will still be applicable unless the patient withdraws consent from further follow up and participation in the trial or if the Sponsor advises that further sampling is not required.

5.4 Duration of treatment

Treatment should continue so that the patient receives six vaccinations unless (a) the patient asks to be withdrawn, (b) there is evidence of disease progression (as per Immune-Related Response Criteria (irRC) given in Appendix 3), (c) the patient is experiencing unacceptable toxicity or (d) the investigator feels the patient should stop the vaccinations for any other reason. Other reasons are listed in Section 11.0.

An End of Vaccination visit will be carried out 30 days post the last vaccination day. This visit will document any emerging AEs since the last vaccination. Adverse events present at this visit, attributed to AST-VAC2 (highly probably, probably or possibly related) will be followed up until resolution or stabilisation of these events (even if the patient starts another anti-tumour treatment).

If a patient terminates treatment prematurely, an End of Vaccination visit should be performed within 30 days of the last vaccination day or when the decision is taken to withdraw the patient from further vaccinations or the trial. These patients should also be asked to continue attending hospital for the 3, 6 and 12 month from first vaccination follow-up visits to provide blood samples for immune analysis (unless the patient withdraws consent from further follow up and participation in the trial or if the Sponsor advises that further sampling is not required).

Patients will be asked to attend follow up as per Section 7.4 until the end of trial (EoT) is declared. The 'End of Trial' will be declared when all patients have either withdrawn (see Section 11.0) from the trial or died, or the last patient has completed their final follow-up visit (5 years post the first vaccination), whichever is the latter.

5.4.1 Replacement of patients

Three administrations of AST-VAC1 (i.e. three complete doses) have previously been shown to demonstrate the first indications of an immune response therefore patients who receive fewer than three AST-VAC2 vaccinations (for reasons other than toxicity) will be replaced, pending vaccine availability.

5.5 Concomitant medication and treatment

Concomitant medication may be given as medically indicated. Details (including name, indication for use and start and stop dates) of the concomitant medication given must be recorded in the patient's medical records and details entered into the eCRF.

The patient must not receive other anti-cancer therapy or investigational drugs during the vaccination phase of the trial. Following the vaccination period, if other treatment is received during the follow up period, this should be documented accordingly however, follow up information (including AEs related to AST-VAC2) should still be collected as per protocol, Section 7.4.

Use of immunosuppressive medication, including corticosteroids (other than replacement dosing for adrenal insufficiency/pituitary failure), is excluded in this protocol. Inhaled and topical steroids are permitted. A short course of systemic steroids may be permitted for management of adverse events, however, this should be discussed and agreed in advance by the CDD Medical Advisor and/or the Chief Investigator.

Patients may be administered a COVID-19 vaccine during the trial (including during screening), at the discretion of the Investigator. Where possible, the COVID-19 vaccination should be administered before

trial entry or after completion of the AST-VAC2 vaccination period. However, if the COVID-19 vaccination is to be administered during the AST-VAC2 vaccination period, there should be an interval of 48 hours between administration of the two vaccines and they should be administered to different sites. Details of the COVID-19 vaccination should be recorded in the medical records and details entered in the eCRF.

6 PHARMACEUTICAL INFORMATION

6.1 Supply of AST-VAC2 vaccine

A complete certificate of analysis and a Qualified Person (QP) certification must be provided with each batch of the AST-VAC2.

For information on AST-VAC2 and re-ordering of supplies, contact the Clinical Research Associate (CRA)/Clinical Study Manager (CSM) responsible for the trial who will arrange further supplies.

AST-VAC2 will be supplied by:

The Cancer Research UK Biotherapeutics Development Unit (BDU) must send a copy of the drug shipment form to the CSM/CRA following despatch of AST-VAC2.

The primary and secondary packaging for AST-VAC2 will be labelled according to Eudralex Volume 4: Annex 13 'Investigational Medicinal Products' of the European Union guide to Good Manufacturing Practice (GMP).

Prior to despatch of AST-VAC2 to the clinical trial site, a label detailing the investigator name and investigator site name will be added to the secondary packaging (where applicable) at the manufacturing authorisation holder (MIA) (IMP) licensed manufacturer in accordance with GMP.

An example of the approved label(s) can be found in the Trial Master File (TMF) and site Pharmacy File.

6.2 Pharmaceutical data

6.2.1 Formulation of AST-VAC2

6.2.2 Storage conditions

All supplies must be stored in a secure, limited access storage area. AST-VAC2 must be stored at ≤-140°C in the vapour phase of a liquid nitrogen tank. The storage temperature must be fully monitored at all times.

6.2.3 Method of dilution/reconstitution of AST-VAC2

There is no reconstitution or dilution of the product required. The IMP should be administered to the patient within a maximum of two hours following removal from storage and thaw. Instructions on thawing and loading the syringe will be supplied to sites.

6.2.4 Stability and labelling of the AST-VAC2

The prepared solution for administration has a maximum time period of two hours from removal from storage, thawing and administration to patient. Product should be stored in a cooled container post thawing during this time. Ideally administration should occur as soon as possible after thaw and within two hours maximum

The product is supplied in Crystal Zenith (CZ) vials packed into a CZ box containing six individual doses and must not be removed from the primary packaging until time of administration. It must not be placed in any other container.

Labelling requirements for the AST-VAC2 must include the following information:

- Cancer Research UK,
- AST-VAC2,
- Batch XXX.XX.XX,
- For Intra-Dermal admin,
- Protocol No CRUKD/17/003,
- 0.6 mL at 2.0 x 10^7 cells/vial

Expiry date will not be detailed on the vial but will be shown on the secondary packaging (CZ boxes).

The actual cell dose patients may receive will vary, up to a maximum of 2 x10^7 viable cells, to achieve a target dose of 1 x 10^7 viable cells - please see Section 6.2.5.

6.2.5 AST-VAC2 administration

Before administering the AST-VAC2 vaccine, the exact dosage must always be double-checked by a second suitably qualified person at site. All checks and double-checks must be documented (signed and dated) and the documentation must be available for the CRA/CSM to verify.

Each weekly AST-VAC2 vaccine dose will be administered as a split dose via two id injections at a target dose defined as 1 × 10^7 viable cells. Each vial of AST-VAC2 will be filled at a concentration of 2 x 10^7 viable cells per vial (pre-cryopreservation) and the release specification for the product strength has been set at $\geq$1 x 10^7 viable cells/vial post thawing (based on stability data). The actual cell dose patients may receive will vary, up to a maximum of 2 x10^7 viable cells, to achieve a target dose of 1 x 10^7 viable cells. There is no dose escalation planned during CRUKD/17/003.

Each vial of AST-VAC2 will contain 0.6 mL. For each vaccination, vial contents should be drawn into one needle from the vial and delivered as two separate id injections. Each injection will be a maximum of 0.3 mL each. The injections should be administered to the same body site approximately 1 cm apart.

Patients should receive the id injections in either the deltoid muscle on the arm, the outer thigh surface, anterior abdominal wall or buttock. If an injection site reaction (ISR) has resolved after the first vaccination day/previous vaccination days then for subsequent injections, injections can be administered at the same body site. If an ISR from a previous vaccination is still present ($\geq$Grade 1) then the injection should be administered into an alternative site. The site of injection will be documented for each vaccination.

If a patient is administered a COVID-19 vaccination during the AST-VAC2 vaccination period, there should be an interval of 48 hours between the administration of the vaccines and they should be administered to different sites.

6.2.6 Vein extravasation/accidental spillages

As AST-VAC2 is administered intradermally, vein extravasation should not be a potential risk. Accidental spillages should be dealt with according to hospital policy.

6.3 AST-VAC2 accountability

Accurate records of all AST-VAC2 shipments and vials dispensed must be maintained in the local pharmacy. Once the product is thawed the injection must be administered within two hours. If this cannot occur, the product cannot be re-frozen and must be disposed of as per hospital policy. This must be noted on the inventory for authorisation by CRA/ CSM. This inventory record must be available for inspection at any time by CRAs or CSMs of the Sponsor. AST-VAC2 supplies are to be used only in accordance with this protocol and under the supervision of the Investigator.

Unused product (thawed or frozen) should be returned to pharmacy for destruction. The Investigator undertakes not to destroy any unused or returned AST-VAC2 unless authorised to do so by the Sponsor. Any unused AST-VAC2 must be destroyed according to hospital procedures and properly accounted for using the IMP Destruction Form and also on the IMP Accountability Record. During the course of the trial the CRA will check the numbers of vials of AST-VAC2 shipped to the centre, the number used and the number destroyed. The pharmacy will give an account of any discrepancy.

7 INVESTIGATIONS SCHEDULE

In cases where a patient has investigations at a different hospital, for example weekly blood samples, scans and other investigations as appropriate, then it is the Investigator's responsibility to ensure he/she receives and reviews the reported results. These results must be available for source data verification. Laboratory reference ranges, including effective dates, and evidence of laboratory accreditation must be obtained from all laboratories used. For scan results, the original images and reports must be available for comparison to any scan performed at the investigator site and be in a format that is suitable for comparison. For all other investigations, apart from the results, any supporting data must also be made available for source data verification or review.

The Investigator or delegate must inform the Sponsor of any changes to their laboratory normal ranges or to any laboratory accreditation and provide any new documentation.

7.1 Pre-treatment evaluations

Details of all evaluations/investigations for enrolled patients, including relevant dates, required by the protocol must be recorded in the medical records.

At the first visit to the participating clinical site and identified as a potential patient by the clinical team, patients will be asked to consent to a blood sample for HLA typing analysis. As HLA analysis and confirmed type is an inclusion criterion, patients who do not consent to this analysis will be unable to enter the trial. It will be clear in the accompanying informed consent document (ICD) that agreement to the HLA analysis does not mean that entry into the main trial is confirmed but that it is one of several screening procedures. If the patient consents, the blood sample will be taken at that visit.

7.1.1 Obtaining written informed consent

Written informed consent must be obtained from the patient before any protocol-specific procedures are carried out.

7.1.1.1 Pre-screening: HLA testing

All potential patients will be asked to consent to HLA testing prior to entering main trial screening. The result of the HLA testing must be known before the patient is introduced to the main patient information sheet. A three mL blood sample will be taken for central lab analysis. Only HLA A*02:01 positive patients will be asked for consent to enter trial screening for the main trial.

The result of HLA testing must be known and the patient must have signed the separate main trial consent before the patient enters the main trial screening stage.

[redacted]

7.1.1.2 Main trial consent and screening

HLA A*02:01 positive patients:

The patient must be given adequate time to think about their commitment to the trial and whether they would agree to being enrolled onto the trial to receive trial treatment. If consent was taken more than 28 days prior to the first AST-VAC2 vaccination then it is the responsibility of the Investigator to determine if further consent should be taken. If there has been a change to the ICD, then the patient must be made aware of the changes made and reasons for the change(s) and consent must be taken again.

For all patients:

Only the Principal Investigator (PI) and those Sub-Investigator(s) with delegated responsibility by the PI, and who have signed the Delegation Log, are permitted to obtain informed consent from patients and sign the consent form. All signatures must be obtained before the occurrence of any medical intervention required by the protocol (ICH GCP 4.8.8 and 8.3.1.2). The patient should sign and date the consent form in the presence of the Investigator, followed by the Investigator signature. The date of the

signatures of both the patient and the PI/Sub-Investigator obtaining informed consent should be the same.

The PI or the Sub-Investigator must inform the patient about the background to their disease and the normal management of their disease alongside information about the AST-VAC2 trial. The investigator must also ensure that the patient is aware of the following points:

- That AST-VAC2 is new and that the exact degree of activity is at present unknown, but that treating him/her will contribute to further knowledge.
- Any known toxicity of AST-VAC2 and the possibility of experiencing side-effects.
- The potential dangers of becoming pregnant (or the patient's partner becoming pregnant) and he/she has been given information about appropriate medically approved contraception (refer to Section 9.7).
- That he/she may refuse treatment either before or at any time during the trial and that refusal to participate will involve no penalty or loss of benefits to which they are otherwise entitled.
- Whom to contact for answers to pertinent questions about the research and their rights, and also who to contact in the event of a research-related injury.
- That long-term follow-up is required because it is an advanced therapy investigational medicinal product (ATIMP). Consent for long term follow up will be requested at the time of trial enrolment.

A copy of the ICD for the main trial must be given to the patient to keep and the original ICD must be filed in the Investigator Trial File (ITF) (unless otherwise agreed that the original document will be filed in the medical records and a copy kept in the ITF).

7.1.2 Evaluations within 28 days prior to administration of AST-VAC2 (Baseline)

(Day -28 to Pre-vaccination on Day 1)

The following must be performed/obtained **within 28 days before** the patient receives their first AST-VAC2 vaccination (and post confirmation that the patient is HLA A*02:01 positive):

Existing results such as radiological measurements may be used even where these investigations were performed prior to the patient's provision of informed consent for the trial, if they were performed within the required time window.

- Written informed consent (may be taken more than 28 days before, see Section 7.1.1.2).
- Demographic details.
- Medical history including prior diagnosis (histologically or cytologically proven lung cancer), prior treatment, concomitant conditions/diseases and baseline signs and symptoms, concomitant treatment.
- Radiological disease assessments: Radiological measurements (computerised tomography (CT) scan, to cover chest, thorax, abdomen, pelvis, liver) and/or magnetic resonance imaging (MRI) – must be performed **within four weeks before** the patient receives the first dose of AST-VAC2. Tumour measurements must be reported according to Immune Response Related Criteria (irRC) – Appendix 3.
- [redacted]

Note that all AEs, including Serious AEs, must be monitored and recorded in the eCRF from the time the patient consents to any protocol-specific procedure, including consent for HLA typing (see Section 9.3) for further details). If the patient is administered a COVID-19 vaccine during the trial screening period, this should be documented in the eCRF as a concomitant medication and any AEs should be assessed for causality to the COVID-19 vaccination.

7.1.3 Evaluations within 7 days of study enrolment

The following must be performed **within 7 days before** study enrolment:

- Female patients able to have children must have a negative result on a human chorionic gonadotropin (HCG) pregnancy test (serum or urine test is acceptable).
- Complete physical examination;
- Height, weight, WHO PS,
- Vital signs (temperature, seated BP and pulse rate);
- ECG;
- Laboratory tests (blood/urine samples). Laboratory tests relating to specific eligibility criteria must be performed to confirm a patient's eligibility prior to study enrolment:
  - Haematology – haemoglobin (Hb), white blood cells (WBC) with differential count (neutrophils, lymphocytes, platelets and eosinophils).
  - Biochemistry – sodium, potassium, adjusted calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) or aspartate aminotransferase (AST) and C-reactive protein (CRP).
  - CEA sample
  - Urinalysis – pH, glucose, protein and blood.
  - Baseline research bloods (Please see Section 8.0)

Investigators must enrol the patient on the trial once confirmed as eligible (see Section 4.2) and prior to the patient's first AST-VAC2 vaccination.

7.2 Evaluations during the trial

Day of vaccination

- Symptom-directed physical examination: if clinically indicated, a symptom-directed physical examination is to be performed on the day of each vaccination before AST-VAC2 administration.
- WHO PS on the day of each vaccination.
- Vital signs, temperature, seated BP, pulse rate: must be assessed within 15 mins before the vaccination and within 15 mins after the vaccination.
- Adverse events and concomitant medications: At each visit, before each AST-VAC2 vaccination, an assessment of any AE experienced since the previous visit must be made by the Investigator, Research Nurse or suitably qualified member of the Investigator's team.

  The start and stop dates of the AE together with the relationship of the event to AST-VAC2 must be recorded in the medical records.

  All AEs must be graded according to NCI CTCAE Version 4.02 and protocol specific ISR grading system (See Section 9.0 for further details regarding AE reporting requirements and ISR grading system).

  Any concomitant treatment must be recorded in the medical records. (See Section 9.0 for further details regarding AE reporting requirements.)
- COVID-19 vaccines: If a patient is administered a COVID-19 vaccination during the AST-VAC2 vaccination period, there should be an interval of 48 hours between the administration of the vaccines and they should be administered to different sites. The COVID-19 vaccine should be documented in the eCRF as a concomitant medication and any AEs should be assessed for causality to AST-VAC2 and the COVID-19 vaccination.
- Laboratory tests:

  Haematology and biochemistry:

  Week 1 vaccination: laboratory tests must be repeated and reported prior to the first vaccination if the results used for enrolment purposes are >7 days prior to the day of vaccination.

Thereafter, laboratory tests must be repeated weekly during the vaccination phase, up to the Week 6 vaccination. Tests to be repeated prior to the weekly vaccination. Haematology and biochemistry results do not need to be reported and checked before each vaccination is given.

- Haematology: detailed in Section 7.1.3.
- Biochemistry: detailed in Section 7.1.3.
- CEA sample
- Urinalysis: detailed in Section 7.1.3.

- Research sampling (Please see Section 8.0):
  - **Research bloods**: within one hour prior to the 3rd, 4th and 6th vaccination.
  - [redacted]

7.3 Evaluations after vaccination

7.3.1 Two weeks post vaccination visit

Two weeks after the patient has received their last vaccination they should be assessed for:

- Adverse events since the last visit. The start and stop dates of the AE together with the relationship of the event to AST-VAC2 (and COVID-19 vaccination if applicable) must be recorded in the medical records. All AEs must be graded according to NCI CTCAE Version 4.02 and protocol specific ISR grading system (See Section 9.0 for further details regarding AE reporting requirements and ISR grading system).
- Concomitant medications (details should be recorded in the medical records).
- Research bloods.
- [redacted]

7.3.2 End of Vaccination visit

The End of Vaccination visit should be performed 30 days (+/- 5 days) after the last AST-VAC2 vaccination. The following investigations should be performed wherever possible.

- A symptom-directed physical examination including WHO PS, vital signs (temperature, seated BP and pulse rate), weight
- Haematology: detailed in Section 7.1.3.
- Biochemistry: detailed in Section 7.1.3.
- CEA sample
- Urinalysis: detailed in Section 7.1.3.
- ECG (unless otherwise indicated by the patient's condition)
- Assessment of AEs (all AEs should be collected until this visit; after this visit only information on ongoing and new AST-VAC2-related AEs should be collected; see Section 9.3) and
- Review of concomitant medications

- Female patients able to have children must have a human chorionic gonadotropin (HCG) pregnancy test (serum or urine test is acceptable).
- Radiological assessment of disease: This must be repeated at the end of the vaccination schedule. Tumour measurements must be documented according to Immune-Related Response Criteria (irRC). (Refer to Section 10.0). Thereafter, assessment of disease to be performed as per standard clinical practice.

7.4 Follow-up

7.4.1 3, 6, 9 and 12 months post first vaccination

Patients should undergo the following assessments:

- Research blood samples 3, 6 and 12 months (all +/-1 week) post the first vaccination.
- At the above time points, an evaluation of any AST-VAC2 related AEs will also be performed. Assessments can be performed as clinically indicated.
- A further AE evaluation should be performed at 9 months post the first vaccination also. This can be in the form of a phone call to the patient.
- Any concomitant medications relevant to AST-VAC2-related AEs should be recorded.

All new and ongoing AST-VAC2-related AEs should be followed up until resolution or stabilisation, even if the patient goes onto another anti-cancer treatment. SAEs considered related to AST-VAC2 are always reportable regardless of the time elapsed.

7.4.2 15, 18 and 21 month post first vaccination

Evaluation of any new or ongoing AST-VAC2-related AEs should be performed at 15, 18 and 21 months (i.e. quarterly). This can be in the form of a phone call to the patient. Any concomitant medications related to these AEs should also be recorded.

It is acceptable for there to be flexibility around when these quarterly visits take place but this should be checked with the Sponsor who will document the revised visit accordingly.

7.4.3 Two years post first vaccination visit (survival follow up data collection).

All vaccinated patients should be followed up for OS, two years post the first vaccination. Evaluation of new or ongoing AST-VAC2-related AEs should be performed. Any concomitant medications relevant to these AEs should also be recorded. The AE information can be collected via a phone call to the patient. Survival information may be obtained from the patient's medical records.

For survival follow up, sites will be asked to provide the following information if known:

- Date of death or date of last contact if alive.
- Reason for death.
- All post trial anti-cancer treatment for primary disease.
- Date of radiological confirmed relapse(s), if post trial.
- WHO PS at time of relapse.
- Any other medically important information, in the opinion of the investigator.

No further patient trial related visits or assessments are required to collect this survival data. Survival follow up information may cease to be collected based on emerging data showing limited immune response and therefore limited benefit of continuing collection of long term follow up data. This will be discussed and agreed by the Sponsor and CI based on emerging data.

7.4.4 Extended follow up (>2 years to 5 years post first vaccination)

After the 2-year survival follow up has been completed, any patients who are still alive will continue to be followed up every three months by telephone until the end of the trial (unless the patient has died or withdrawn from the trial). If the patient has died, the date of death and cause of death will be recorded. Evaluation of new or ongoing AST-VAC2-related AEs should be performed. Any concomitant medications relevant to these AEs should also be recorded. It is acceptable for there to be flexibility around when these quarterly calls take place but this should be checked with the Sponsor who will document the revised visit accordingly.

7.5 Schedule of events

Observation/Investigation	Pre-screening & trial screening	Within 7 days before study enrolment	During the trial			Two weeks post last vaccination	End of Vaccination visit 30 days after last vaccination	Research bloods: Up to 12 months post first vaccination [g] Survival follow up: Up to 2 years post first vaccination [f]	Extended follow up: >2to 5 years post first vaccination (visits conducted by telephone)
			Vaccination at Weeks 1, 2, 3, 4, 5 and 6						
	Day -28 to -1 prior to first vaccination		Day -1 or Day of vaccination	Pre AST-VAC2 Within 15min prior	Post AST-VAC2 15 mins				
Written informed consent – HLA typing [a]	X								
Written informed consent– Main Trial [b]	X								
Adverse event evaluation [l]	From date of informed consent, throughout trial & monthly follow up thereafter until resolution [j] Ongoing drug-related AEs will be followed up until completion of extended follow up.								X[n]
Medical history	X								
Concomitant treatments	Throughout trial								X[n]
Radiological disease assessment [k]	X						X		
Pregnancy test (if applicable)		X					X		
Physical examination		X	X [m]				X		
Vital signs [c]		X		X	X		X		
WHO Performance Status [d]		X	X				X		
Demographics	X								
Height		X							
Weight		X					X		
Haematology, biochemistry, CEA		X	X [e]				X		
Urinalysis		X	X [e]				X		
Electrocardiogram		X					X		
AST-VAC2 administration			2 x id injections on each vaccination day, administered weekly, 7 days apart (+/- 1 day)						
Research bloods		X	Weeks 3, 4 and 6 pre-vaccination			X		3, 6 and 12 months post first vaccination	
[redacted]									
[redacted]									
Survival follow up [i]								X	

a) Patients will be asked to give written consent for HLA testing before any other further trial screening is performed. Thereafter, consent will be sought for the main trial.

It is acceptable that the HLA testing is performed outside of the 28 day window: important to note that this test will be performed by a central laboratory therefore sites should plan accordingly to ensure that following consent, the sample is taken and shipped to ensure timely reporting in preparation for the proposed first vaccination.

b) HLA A*02:01 positive patients will be asked consider consenting to the main trial for trial treatment and enter the screening period.

c) Vitals to include temperature, seated BP, pulse rate.

d) WHO PS to be assessed on day of vaccination as well as during screening.

e) Haematology, biochemistry, CEA and urinalysis: Week 1 vaccination: laboratory tests must be repeated and reported prior to the first vaccination if the results used for enrolment purposes are >7 days prior to the day of vaccination.
Thereafter laboratory parameters are to be measured on Day-1 or Day of vaccination. Haematology and biochemistry results do not need to be reported and checked before each vaccination is given.

f) Follow up for overall survival to be captured for two years post the first vaccination.

For extended follow up details, see Section 12.0.

g) Research blood sampling required, pre, during and post treatment – Please see Section 8.0.

[redacted]

[redacted]

j) Monthly follow-up required ONLY for those AEs and SAEs considered drug-related (highly probable, probable or possible) that are present at time that patient completes the End of Vaccination visit. Monthly follow-up to continue until resolution, to baseline, stabilisation or patient starts another anti-cancer treatment.

AE evaluation also to be performed as per Section 7.4.

k) Radiological disease assessment prior to trial start may have been taken prior to informed consent as part of routine diagnostic procedures.
Tumour measurements must be reported according to Immune Response Related Criteria (irRC).

l) AE evaluation according to CTCAE v4.02 and protocol specific grading system for ISR.

m) Symptom directed physical examination only.

n) During extended follow up, new and ongoing AST-VAC2 related AEs should be reported and only concomitant medications relevant to these AST-VAC2 related AEs should be recorded.

8 PHARMACODYNAMIC ASSESSMENTS

8.1 Summary of Pharmacodynamic assessments

1) Pre-screening assessment

	Biomarker	Technology	Source	Rationale	Time point	Patient group	Planned timing of analysis
1a	HLA A*02:01	Luminex or similar	Whole blood 3 mL *	Patient eligibility	Screening visit	All patients	Result required prior to further screening assessments

* Analysis from the pre-screening HLA typing will report on several alleles. DNA extracted from the whole blood sample will be stored for further allele analysis as confirmed by the Sponsor, See 3h.

2) Secondary Endpoints

	Biomarker	Technology	Source	Rationale	Time point	Patient group	Planned timing of analysis
2a	hTERT specific T cell ELISPOT analysis	ELISPOT	Whole blood 72 mL at baseline, thereafter, 48 mL to be taken at each time point	Identification of peptide specific T cells	• Baseline • Within one hour prior to 3rd, 4th and 6th vaccination • 2 weeks (+/-1 day) post last vaccination • 3, 6, 12 months (+/- 1 week) post first vaccination	All treated patients	Analysis as defined by the Sponsor.

3) Tertiary endpoints

	Biomarker	Technology	Source	Rationale	Time point	Patient group	Planned timing of analysis
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

For the above table, blood [redacted] samples will be taken at the local participating clinical site for either local storage (until the Sponsor confirms shipping requirements and receiving laboratory) or will be shipped to a central analysing laboratory. The Sponsor will define the local storage requirements prior to shipment or will coordinate the shipment of the sample to the central laboratory. Thereafter, storage of remaining samples will be as per Sponsor agreements with the relevant laboratories.

8.2 Primary assessments

There are no primary endpoint pharmacodynamic assays for this trial.

8.3 Secondary assessments

8.3.1 Pharmacodynamic blood biomarkers (secondary)

Please refer to the AST-VAC2 Laboratory Manual for instructions of collection, handling and storage.

Sample collection schemes may be reconsidered during the trial upon collection of emerging pharmacodynamic data.

- If a patient has received injections on less than three vaccination days, research blood sampling at 3, 6 and 12 months may be omitted based on emerging data. The Sponsor will advise clinical sites so that patient visits can be arranged as required.

- If no immune response is seen at the three month time point, later time points may not be analysed. If the patient consents, any remaining samples not yet analysed will be considered a research 'gift' before future research is performed on the sample.

Blood biomarkers will be measured in whole blood or serum according to agreed standard operating procedures (SOPs) and validated methods.

All patients enrolled will be asked to consent to research bloods and the blood amounts from each patient at the various time points are as follows:

Time point	Total amount, whole blood in mL
Pre-screening (HLA analysis)	3
Baseline*	96
Week 3 vaccination*	69
Week 4 vaccination*	69
Week 6 vaccination*	69
2 weeks post last vaccination*	69
3 months post first vaccination*	75
6 months post first vaccination	66
12 months post first vaccination	66

[redacted]

The approximate total volume of blood withdrawn from each patient for will be 585 mL over a 12 month period.

- 378 mL in first eight weeks
- At 3 months, 75 mL
- At 6 months, 66 mL
- At 12 months, 66 mL.

Sampling time points will be subject to change based on emerging data. The Sponsor will confirm any changes with participating sites and subject to either a substantial/non-substantial protocol amendment, based on the changes made.

8.3.1.1 Identification of peptide specific T cells (Secondary assay 2a)

Whole blood will be collected for the identification of peptide-specific T cells using ELISPOT. A 72 mL sample of whole blood will be collected from all patients at baseline, thereafter, 48 mL will be taken prior to the third, fourth and sixth (or final) vaccinations, 2 weeks after the last vaccination and at 3, 6 and

12 months following the first vaccination. The approximate total volume of blood withdrawn from each patient for this analysis will be 408 mL.

8.4 Tertiary/research assessments

[redacted]

[redacted]

9 ASSESSMENT OF SAFETY

9.1 Investigator Responsibilities

The investigator is responsible for monitoring the safety of patients who have enrolled in the trial and for accurately documenting and reporting information as described in the following sections.

9.1.1 Medical Cover

The chief/principal investigator (CI/PI) is also responsible for ensuring patients have access to 24 hour advice and/or care. Patients will be provided with the necessary contact numbers for both normal working and out of hours care. A copy of the protocol must be made available out of hours to ward staff and clinicians on call so that the appropriate advice may be given to the patient, the patient's relative or other care giver (for example general practitioner (GP)). The CI/PI must ensure that should the on call clinician or ward staff require more advice than is in this protocol, that they have access to the Investigator or delegated members of the investigator's team who can answer any questions.

9.2 Adverse event definitions

9.2.1 Adverse event

Adverse events for this trial will be graded according to NCI CTCAE version 4.02. In addition, Injection Site Reactions will be graded according to protocol specific grading system as per Section 9.8.

An adverse event (AE) is any untoward, undesired or unplanned medical occurrence in a patient administered an IMP, a comparator product or an approved drug.

An AE can be a sign, symptom, disease, and/or laboratory or physiological observation that may or may not be related to the IMP or comparator.

An AE includes but is not limited to those in the following list.

- A clinically significant worsening of a pre-existing condition. This includes conditions that may resolve completely and then become abnormal again.
- AEs occurring from an overdose of an IMP, whether accidental or intentional.
- AEs occurring from lack of efficacy of an IMP, for example, if the Investigator suspects that a drug batch is not efficacious or if the Investigator suspects that the IMP has contributed to disease progression.

9.2.2 Serious adverse events

A serious adverse event (SAE) is any AE, regardless of dose, causality or expectedness, that:

- results in death;
- is life-threatening*;
- requires in-patient hospitalisation *or* prolongs existing in-patient hospitalisation (some hospitalisations are exempt from SAE reporting – e.g. hospital admissions planned prior to the patient entering the trial; overnight stays for planned procedures such a blood transfusions (Section 9.4.1);
- results in persistent or significant incapacity or disability;
- is a congenital anomaly or birth defect;
- is any other medically important event**

* A life-threatening event is defined as an event when the patient was at substantial risk of dying at the time of the adverse event, or use or continued use of the device or other medical product might have resulted in the death of the patient.

** A medically important event is defined as any event that may jeopardise the patient or may require intervention to prevent one of the outcomes listed above. Examples include allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias

(blood disorders) or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of important medical events.

For fatal SAEs, wherever possible report the cause of death as an SAE with a fatal outcome rather than reporting death as the SAE term. When available, the autopsy report will be provided to the Sponsor.

If during the course of the trial, other medically important events are identified and there is a requirement to report specific events outside of the standard criteria, this will be communicated to sites and the protocol will be updated to reflect this.

Other reportable events that must be treated as SAEs are listed below.

- Any AST-VAC2 related adverse event which meets the stopping rule criteria (as outlined in Section 3.3.3) are considered a trial specific medically important events and therefore an SAE.
- Pregnancy exposure to the IMP. Any pregnancy occurring in a patient or a patient's partner during treatment with an IMP or occurring within six months of the last IMP administration, must be reported to the Pharmacovigilance Department in the same timelines as an SAE. These should be reported even if the patient is withdrawn from the trial.
- Overdose with or without an AE.
- Inadvertent or accidental exposure to an IMP with or without an AE, including for example, spillage of the IMP that contaminates staff.
- Any AE that could be related to the protocol procedures, and which could modify the conduct of the trial.

9.2.3 Suspected, unexpected, serious adverse reactions

A SUSAR is a suspected, unexpected, serious adverse reaction. All AEs and SAEs will be assessed by the Sponsor for seriousness, causality and expectedness. The Pharmacovigilance Department will expedite all suspected unexpected serious adverse reactions (SUSARs) to the relevant Competent Authority/Authorities and the relevant Ethics Committee(s) within the timelines specified in legislation (SI 2004/1031 as amended).

9.2.4 Determining adverse event causality

The relationship of an AE to the IMP is determined as follows.

Highly probable

- Starts within a time related to the IMP administration and
- No obvious alternative medical explanation.

Probable

- Starts within a time related to the IMP administration and
- Cannot be reasonably explained by known characteristics of the patient's clinical state.

Possible

- Starts within a time related to the IMP administration and
- A causal relationship between the IMP and the AE is at least a reasonable possibility.

Unlikely

- The time association or the patient's clinical state is such that the trial drug is not likely to have had an association with the observed effect.

Not related

- The AE is definitely not associated with the IMP administered.

Note: Drug-related refers to events assessed as possible, probable or highly probable.

The Investigator must endeavour to obtain sufficient information to determine the causality of the AE (i.e. IMP, other illness, progressive malignancy etc.) and must provide his/her opinion of the causal relationship between each AE and IMP. This may require instituting supplementary investigations of significant AEs based on their clinical judgement of the likely causative factors and/or include seeking a further opinion from a specialist in the field of the AE.

The following guidance should be taken in to account when assessing the causality of an AE:

- Previous experience with the IMP and whether the AE is known to have occurred with the IMP.
- Alternative explanations for the AE such as concomitant medications, concurrent illness, non-medicinal therapies, diagnostic tests, procedures or other confounding effects.
- Timing of the events between administration of the IMP and the AE.
- De-challenge, that is, if the IMP was discontinued or the dosage reduced, what happened to the adverse reaction?
- Re-challenge, that is, what happened if the IMP was restarted after the AE had resolved?

9.2.5 Expectedness

Assessment of expectedness for AST-VAC2 will be made by the Pharmacovigilance Department against Reference Safety Information in the current version of the IB, current at the time of the event.

9.3 Collection of safety information

9.3.1 Screening failures

For patients who fail screening, SAEs must be reported to the Pharmacovigilance Department, CDD from the date of consent until the date the patient is confirmed as ineligible.

9.3.2 Patients who consent to HLA screening

AEs and SAEs related to the HLA test procedure should be reported from the time of HLA test consent to the time of consent to the main trial. Thereafter, should the patient consent to the main trial, reporting requirements as per Section 9.3.4.

9.3.3 Eligible patients

For eligible patients, SAE and AE collection and monitoring will commence at the time the patient gives their written consent to participate in the trial by signing the main trial consent form and will continue until 30 days after the last vaccination (administration of IMP). From this time point until the patient withdraws, dies or completes the trial, new or ongoing AST-VAC2-related AEs will be collected (regardless of whether the patient goes on to other anti-cancer therapies).

Should an Investigator become aware of any AST-VAC2 -related SAEs after the patient has completed the protocol-mandated follow-up period (as specified above), these must also be reported to the Sponsor within the expedited timelines in Section 9.4.

9.3.4 Follow-up of AEs and SAEs

Follow-up of AEs with a causality of possible, probable or highly probable will continue until the events resolve, stabilise or the patient starts another anti-cancer therapy.

The Pharmacovigilance Department will make requests for further information on SAEs to the trial site at regular intervals. Requested follow-up information should be reported to the Pharmacovigilance Department in a timely manner and as soon as possible after receipt of the follow-up request. For fatal or life-threatening cases, follow-up information must be reported to the Pharmacovigilance Department as soon as possible.

9.3.5 Other safety information of interest

We will also collect information on the following situations, whether they are associated with an AE or not:

- Abuse or misuse.

- Occupational exposure (to a person other than the patient, for example spilling of IMP on hands of nurse or splashing in the eye).

Any occurrences of these should be reported in the same manner as SAEs (Section 9.4).

9.4 Reporting of SAEs to the Sponsor Pharmacovigilance Department

All SAEs, regardless of causality, must be reported to the Pharmacovigilance Department in an expedited manner.

SAEs should be documented on an SAE report form, using the completion guidelines provided.

The SAE report form should be e-mailed to Pharmacovigilance Department within 24 hours of site staff becoming aware of the SAE.

[redacted]

Each episode of an SAE must be recorded on a separate SAE report form. The NCI CTCAE Version 4.02 and protocol specific grading system for injection site reactions, must be used to grade the severity of each SAE, and the worst grade recorded. If new or amended information on a previously reported SAE becomes available, the Investigator should report this to the Pharmacovigilance Department on a new SAE report form.

If the SAE has not been reported within the specified timeframes, a reason for lateness must be added to the email when sending the SAE report form to the Pharmacovigilance Department.

Should the Investigator become aware of any AST-VAC2-related SAEs after the patient has withdrawn from the trial, these must also be reported to the PV Department within the specified timelines specified above.

9.4.1 Events exempt from being reported as SAEs to the Pharmacovigilance Department

Events specified in this section do not require reporting as SAEs in this trial, unless hospitalisation is prolonged for any reason and then an SAE form must be completed. The events must still be recorded in the appropriate section of the eCRF.

Elective admissions – Elective admissions to hospital for procedures which were planned prior to entering the trial are not SAEs. Hospitalisation for administration of the IMP according to the trial protocol is also exempt from being reported as an SAE.

Prolongation of hospitalisation - Prolongation of hospitalisation without an associated adverse event (for example, prolonged hospitalisation while appropriate social care is set up for elderly patients).

Death due to disease progression- Cases of death due to disease progression do not require SAE reporting, unless considered related to the IMP.

9.5 Recording of adverse events and serious adverse events in eCRFs

All AEs, including SAEs, must be recorded in the eCRF for eligible patients. All concomitant medications, including herbal medications and supplements must be recorded. Any therapy used to treat the event must be recorded. The eCRF will be reconciled with the safety database during and at the end of the trial. Therefore, the sites should ensure the data entered on the SAE report form and the data entered into the eCRF are consistent. The Sponsor's Medical Advisor and the Investigator(s) will regularly review the safety data from both the safety and the clinical database.

9.6 Urgent safety measures

The Sponsor or Investigator may take appropriate urgent safety measures (USMs) in order to protect the patient of a clinical trial against any immediate hazard to their health or safety. This includes procedures taken to protect patients from pandemics or infections that pose serious risk to human health.

USMs may be taken without prior authorisation from the competent authority.

The Medicines and Healthcare products Regulations Agency (MHRA) and the Research Ethics Committee (REC) must be notified within three days of such measures being taken.

Should the site initiate a USM, the Investigator must inform the Sponsor immediately either by:

[redacted]

The notification must include:

- the date of the USM;
- who took the decision; and
- why action was taken.

The Sponsor will then notify the MHRA and the REC within three days of USM initiation.

9.7 Pregnancy

Female patients who become pregnant during the trial treatment period must be withdrawn from trial treatment immediately.

The Investigator must make every effort to try and ensure that a clinical trial patient or a partner of a clinical trial patient does not become pregnant during the trial or for six months afterwards. This should be done as part of the consent process by explaining clearly to the patient the potential dangers of becoming pregnant and also providing each patient with information about appropriate medically approved contraception. Two forms of medically approved contraception should be used, such as:

- oral contraceptives and condom; (oral, injected or implanted hormonal contraceptives should be used for four weeks before the patient joins the trial)
- intra-uterine device (IUD) and condom;
- diaphragms with spermicidal gel and condom.

Contraception should be effective before the patient is enrolled on the trial, throughout the trial and for six months after completing the trial.

Alternatively the patient may agree to sexual abstinence, effective from the first administration of *IMP*, throughout the trial and for *six* months afterwards. Abstinence is only considered to be an acceptable method of contraception when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

It should be explained to the patient that if his partner is pregnant or breast-feeding when he is enrolled on the trial, the patient should use barrier method contraception (condom plus spermicidal gel) to prevent the unborn baby or the baby being exposed to AST-VAC2.

However, if a patient or a partner of a patient does become pregnant, the reporting procedures below must be followed.

Any pregnancy occurring in a patient or a patient's partner during treatment with an IMP or occurring within six months of last IMP administration must be reported to the Pharmacovigilance Department within 24 hours of the site staff becoming aware of it using a Pregnancy Report Form (provided in the ITF). It is the Investigator's responsibility to obtain consent for follow-up from the patient or patient's

partner. In addition, the Investigator must be made aware of the need to obtain contact details for the patient's partner's GP. The Pharmacovigilance Department will follow-up all pregnancies for the pregnancy outcome via the Investigator, using a Pregnancy Report Form.

The Investigator should document within the patient notes, that the patient confirms consent for the Sponsor to collect pregnancy follow-up information. In the case that the partner of a patient becomes pregnant, a consent form should be provided to the patient's partner in order to obtain consent for collecting privacy data, in accordance with the data protection act.

The Investigator must ensure that all patients are aware at the start of a clinical trial of the importance of reporting all pregnancies (in themselves and their partners) that occur whilst being treated with the IMP and occurring up to six months after the last IMP administration. The Investigator should offer counselling to the patient and/or the partner, and discuss the risks of continuing with the pregnancy and the possible effects on the foetus. Monitoring of the patient should continue until the conclusion of the pregnancy, if the patient or patient's partner has consented to this. Monitoring of the baby should continue until 12 months after birth, if the patient or patient's partner has consented to this.

9.8 Protocol specific grading: Injection Site Reactions

Grade 1	Grade 2	Grade 3	Grade 4
Tenderness with or without: **Itching** **Erythema** **Swelling** **Ulceration** (fluid vesicle or ulcer <10mm) **Minimal effect on activities of daily living.**	**Pain with or without:** **Erythema** **Swelling** **Phlebitis** **Lipodystrophy** **Oedema** **Ulceration** (blood vesicle or ulcer ≥10mm) **Restricts activities of daily living**.	**Severe tissue damage; operative intervention indicated** **Prevents or severely limits activities of daily living**.	**Life-threatening consequences; urgent intervention indicated**

10 ASSESSMENT OF EFFICACY

Response will be assessed to measure any potential responses to trial treatment.

Disease will be measured according to the modified Immune-Related Response Criteria (irRC) given in Appendix 3.

10.1 Timing and type of tumour assessments

All patients will have been evaluated by radiological (CT, MRI) disease assessment as part of their routine care and prior to trial entry. A pre-treatment, baseline assessment must be performed within 28 days of the planned first vaccination. This can be an assessment performed specifically for the trial or may be an assessment which was performed as per clinical practice which complies with the 28 day timeframe for baseline assessments.

All patients that receive at least one AST-VAC2 vaccination will be further evaluated following their last vaccination by the imaging modality performed at baseline.

Index and non-index lesion(s) should be selected from the pre-treatment scan. Index lesions are measurable (≥5x5 mm) and non-index lesions are non-measurable (<5x5 mm).

At baseline, the sum of products of the two largest perpendicular diameters (SPD) of all index lesion(s) is calculated. At the post-treatment tumour assessment, the SPD of the index lesion(s) and of any new measurable lesions (≥5x5 mm) are added together to provide the total tumour burden.

All tumour measurements must be clearly documented on the pre and post treatment scan reports.

Copies of the scans (including those taken pre-trial) must be available for external independent review if requested by the Sponsor.

10.2 Tumour response

All patients who meet the eligibility criteria, receive at least one AST-VAC2 vaccination, have a baseline assessment and at least one repeat assessment of measurable disease will be evaluable for response. Response assessment will be performed in accordance with irRC by defining the change in total tumour burden relative to baseline measurements (Appendix 3). Response categories as per Appendix 3. Should rapid tumour progression occur before the completion of the scheduled six AST-VAC2 vaccinations the patient will be classified as having early progression (EP).

Expert reviewers appointed by the Sponsor may undertake an independent review of the Investigator's assessed objective responses immune response complete response (irCR) and immune response partial response (irPR). Any independent reviewer's assessment will also be documented in the final Clinical Study Report (CSR) along with the original assessment made by the Investigator. The eCRF will reflect the Investigator's opinion.

10.2.1 Recording of response in the eCRF

All pre and post treatment tumour measurements and an applicable overall response category must be recorded in the eCRF.

11 PATIENT WITHDRAWAL BEFORE COMPLETION OF TREATMENT SCHEDULE

AST-VAC2 has been classified as an ATIMP therefore extended follow up (up to five years post first vaccination) is applicable for all patients who receive the IMP.

The Investigator must make every reasonable effort to keep each patient on trial for the whole duration of the trial in order to collect long term safety and pharmacodynamic data. However, if the Investigator removes a patient from the trial (prior to completion of the vaccinations) or if the patient declines further participation, final assessments (as per the 'End of Vaccination' visit), should be performed ideally before any subsequent therapeutic intervention. All the results of the evaluations and observations, together with a description of the reasons for withdrawal from the trial, must be recorded in the patient's medical records and in the eCRF.

A patient may stop vaccination due to AEs but would not be withdrawn from the trial and would continue for follow-up of safety, survival and tertiary endpoints unless they withdraw.

The following are justifiable reasons for the Investigator to withdraw a patient from the trial.

- Withdrawal of consent.
- Serious deviation from the trial protocol (including persistent patient attendance failure and persistent non-compliance).
- Sponsor's decision to terminate the trial.
- Withdrawal by the Investigator for clinical reasons not related to AST-VAC2.
- Loss to follow up.

12 DEFINING THE END OF TRIAL

The 'End of Trial' is defined as the date when all patients have either withdrawn (see Section 11.0) from the trial or died, or the last patient has completed their final follow-up visit (whichever is the latter). The final follow-up visit is scheduled to take place five years after the patient's first vaccination.

It is the responsibility of the Sponsor to inform the MHRA and the REC within 90 days of the End of the Trial that the trial has closed.

In cases of early termination of the trial (for example, due to toxicity) or a temporary halt by the Sponsor, the Sponsor will notify the MHRA and the REC within 15 days of the decision and a detailed, written explanation for the termination/halt will be given.

Recruitment will cease when:

- The drug is considered too toxic to continue treatment before the required number of patients have been recruited.
- The stated number of treated patients to be recruited has been reached.
- The stated objectives of the trial have been achieved.

Regardless of the reason for termination, all data available for patients at the time of discontinuation of follow-up must be recorded in the eCRF. All reasons for discontinuation of treatment must be documented.

In terminating the trial, Sponsor and the Investigators must ensure that adequate consideration is given to the protection of the patient's interests.

13 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Further development of the AST-VAC2 program will consider the following factors:

- Demonstration that vaccination administration is feasible with an acceptable safety profile,
- Demonstration of durable immune response as per the trial secondary endpoint,
- Demonstration of an increase in OS.

13.1 Interim analysis

An interim analysis will be conducted once all patients administered AST-VAC2 have withdrawn, died or completed their two-year follow-up visit for survival.

Once this condition is met, a data cut-off will be established. All patient visits occurring on or before this date will be analysed and summarised in the clinical study report including safety data to date and assessment of overall survival at two years.

Any data collected after this date will be summarised in a supplemental report produced for the final analysis to satisfy the safety reporting requirements for AST-VAC2 as an ATIMP.

13.2 Final analysis

The final analysis will be conducted after one of the following conditions is met:

- The trial is terminated early e.g. due to toxicity
- The End of Trial as defined in Section 12.0 has been reached.

Once one of the conditions is met, a data cut-off date will be established. All patient visits occurring on or before this date will be analysed and summarised in a supplemental report.

13.3 Presentation of data

Data will be presented in a descriptive fashion. Variables will be analysed to determine whether the criteria for the trial conduct are met. This will include a description of patients who did not meet all the eligibility criteria, an assessment of protocol deviations, IMP accountability and other data that impact on the general conduct of the trial.

Baseline characteristics will be summarised for all enrolled patients. Patients who died or withdrew before treatment started or did not complete the required safety observations will be described and evaluated separately.

Treatment administration will be described for all cycles. Vaccine administration, modifications or delays and the duration of treatment will be described.

13.4 Safety

Safety data will be collected from the date of written consent until patients have completed their required follow up period. Safety variables will be summarised by descriptive statistics. Laboratory variables will be described using the NCI CTCAE Version 4.02 and the protocol specific grading system for ISRs.

Adverse events will be reported as tables of frequency of AEs by body system and by worse severity grade observed. Tables should indicate related and unrelated events.

Deaths reported within 30 days of the last vaccination and SAEs will be described separately.

A safety review committee, consisting of all investigators and representatives of the Sponsor, will review emerging safety data on an ongoing basis, with particular reference to ensuring that patients are able complete the vaccination schedule without AST-VAC2 toxicity-related delays. Should the committee

have significant concerns about toxicity seen during the trial they will have the authority to suspend or terminate the trial and/or make recommendations to the Sponsor to modify vaccination administration.

13.5 Pharmacodynamics

The pharmacodynamic analyses to be performed are described below. Reports will undergo a quality control (QC) step prior to finalisation and will be signed by the person responsible for performing the assays and the laboratory quality assurance (QA) manager once final.

Pharmacodynamic biomarker	Analysis plan
HLA status	HLA A*02:01 status to confirm eligibility is a pre-screening assessment and will be performed by Luminex or similar method at a central laboratory. The whole A* allele will be reported during this pre-screening assessment. [redacted]
hTERT specific T cell ELISPOT analysis	Vaccine induced ELISPOT response in blood will be reported longitudinally for each patient. Analysis as defined by the Sponsor.
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

13.6 Tumour response

Disease assessment will be performed at baseline and following completion of the vaccination schedule for measurement of potential responses to trial treatment. Patients who receive at least one vaccination and have both a baseline and at least one repeat assessment, will be evaluable for response.

Objective responses and the best tumour response achieved by each patient while on trial and overall survival will be described and presented in data listings.

Overall survival data will continue to be collected (at those time points noted in Section 7.4) for up to two years after the first vaccination for all patients or until all patients have died, according to whichever event occurs first.

14 ADMINISTRATION

This trial is conducted under a Clinical Trial Authorisation (CTA) and approval from the MHRA, HRA and the relevant REC will be obtained before the start of this trial. This trial is sponsored and monitored by the Cancer Research UK, CDD. Applicable regulatory requirements are described in this section.

14.1 Protocol deviations and amendments

The protocol should be adhered to throughout the conduct of the trial, if a situation arises where the conduct of the trial may not be in line with the protocol, then the site should contact the Sponsor to discuss this.

Amendments to the protocol may only be made with the approval of the Sponsor. A protocol amendment may be subject to review by the assigned REC, HRA and the MHRA. Written documentation of the REC, HRA (and if appropriate the MHRA) 'favourable opinion' (i.e. approval) must be received before the amendment can be implemented and incorporated into the protocol if necessary.

14.2 Serious breach of Good Clinical Practice

A serious breach is a breach which is likely to effect to a significant degree: the safety or physical or mental integrity of the subjects of the trial, or the scientific value of the trial.

In order that the Sponsor can fulfil their obligations in terms of reporting serious breaches of GCP to the MHRA within seven calendar days of identification, site staff must inform the Sponsor of any unplanned deviations to the trial protocol (or GCP principles) as soon as possible after the deviation occurs to allow prompt evaluation by the Sponsor.

14.3 Completion of the electronic case report form

Electronic CRFs approved by the Sponsor will be used to collect the data. The Investigator is responsible for ensuring the accuracy, completeness, clarity and timeliness of the data reported in the eCRFs.

Only the Investigator and those personnel who have signed the Delegation Log provided by the Sponsor and have been authorised by the Investigator should enter or change data in the eCRFs. Authorised users will be included on a user list in order to be provided access to the eCRF. All protocol required investigations must be reported in the eCRF. The Investigators must retain all original reports, traces and images from these investigations for future reference.

Amendments to eCRF data will be made directly to the system and the system audit trail will retain details of the original value(s), who made the change, a date and time, and a reason for the change.

Once an eCRF form has been entered by the site personnel, the data are cleaned using manual and automated checks. Queries will be issued electronically to the site. Authorised personnel must answer the queries by making relevant amendments to data or providing a response. Answered queries will be closed or reissued as appropriate.

Once the patient has completed or withdrawn from the trial and the eCRF has been fully completed, the Investigator must provide an electronic signature to authorise the complete subject casebook. At the end of the trial all eCRFs are retained and archived by the Sponsor and a portable document format (PDF) copy provided to the Investigator who is responsible for archiving at site.

The collection and processing of personal data from the patients enrolled in this clinical trial will be limited to those data that are necessary to investigate the efficacy, safety, quality and usefulness of the trial drug used in this trial. The data must be collected and processed with adequate precautions to ensure patient confidentiality and compliance with applicable data privacy protection according to the applicable regulations. The data collected will comply with Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data.

14.4 Trial performance, monitoring, auditing and inspection

Before the trial can be initiated, the prerequisites for conducting the trial must be clarified and the organisational preparations made with the trial centre. The Sponsor must be informed immediately of any change in the personnel involved in the conduct of the trial.

During the trial the Sponsor's CRA will be responsible for monitoring data quality in accordance with the Sponsor's SOPs. A strategic monitoring approach, including targeted source data verification (SDV), will be implemented where appropriate.

Before the trial start, the Investigator will be advised of the anticipated frequency of the monitoring visits. The Investigator will receive reasonable notification before each monitoring visit.

It is the responsibility of the CRA to:

- review trial records and compare them with source documents;
- check pharmacodynamic samples and storage;
- discuss the conduct of the trial and the emerging problems with the Investigator;
- check that the drug storage, dispensing and retrieval are reliable and appropriate; and
- verify that the available facilities remain acceptable.

At the end of the trial all unused AST-VAC2 supplied must be destroyed at site (only once authorised to do so by the CRA or CSM) or if authorised by the Sponsor returned to the supplier.

It is the responsibility of the Sponsor to notify the MHRA and REC of the EoT. (See definition in Section 12.0).

During the course of the trial, the QA Department of the Sponsor, or external auditors contracted by the Sponsor, may conduct an on-site audit visit (ICH Topic E6 (R1) Guideline for Good Clinical Practice Sections 1.6).

Principal Investigators conducting this trial will accept the potential for inspection by the MHRA.

14.5 Source document verification

Unless agreed in writing, all data collected in the eCRF must be verifiable by the source data. Therefore it is the Investigator's responsibility to ensure that both he/she and his/her trial team records all relevant data in the medical records. The Investigator must allow the CRA direct access to relevant source documentation for verification of data entered into the eCRF, taking into account data protection regulations. Entries in the eCRF will be compared with patients' medical records and the verification will be recorded in the eCRF.

Some source data may exist only electronically and be entered, or loaded directly into the eCRF.

The patients' medical records, and other relevant data, may also be reviewed by appropriate qualified personnel independent from the Sponsor appointed to audit the trial, National Health Service (NHS) Trust staff and by regulatory authorities. Details will remain confidential and patients' names will not be recorded outside the hospital.

14.6 Clinical study report

At appropriate intervals, interim data listings will be prepared to give the Investigator the opportunity to review the data and check the completeness of information collected. All clinical data available will be presented at the interim analysis and at the end of the trial on final data listings. The Sponsor will prepare a CSR based on the interim final data listings and a supplemental report based on the final data listings at the end of the trial. The CSR and supplemental reports will be submitted to the Investigator(s) for review and confirmation that it accurately represents the data collected during the course of the trial. Summary results of the interim and final analysis of the trial will be provided by the Sponsor to the MHRA and to the REC.

14.7 Record retention

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable both the conduct of a clinical trial and the quality of the data produced to be evaluated and verified. These essential documents (as detailed in Chapter V of Volume 10 (Clinical Trials) of The Rules Governing Medicinal Products in the European Union based upon Section 8 of the ICH GCP Guidelines), including source documents such as scans, trial related documents and copies of the eCRFs, associated audit trail and SAE report forms, shall show whether the Investigator has complied with the principles and guidelines of GCP.

All essential documents required to be held by the Investigator must be stored in such a way that ensures that they are readily available, upon request, to the Regulatory Agency or Sponsor, for the minimum period required by national legislation or for longer if needed by the Sponsor. Records must not be destroyed without prior written approval from the Sponsor.

The medical files of trial subjects shall be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

14.8 Ethical considerations

Before starting the trial, the protocol and patient ICDs must go through the Sponsor's external review process, and be approved by the Protocol and Safety Review Board (PSRB) and receive the favourable opinion of the assigned REC.

It is the CI/PI's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the trial. The CI/PI must ensure this is documented in the patient's medical notes and the patient is re-consented.

The Sponsor and CI/PI must ensure that the trial is carried out in accordance with the GCP principles and requirements of the UK Clinical Trials regulations (SI 2004/1031 and SI 2006/1928 as amended), the ICH GCP guidelines and the World Medical Association (WMA) Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and all subsequent amendments including Oct 2013.

14.9 Indemnity

This trial is sponsored by Cancer Research UK and therefore injury to a patient caused by the compounds under trial will not carry with it the right to seek compensation from the pharmaceutical industry. Cancer Research UK will provide patients with compensation for adverse side effects, in accordance with the principles set out in the Association of the British Pharmaceutical Industry (ABPI) guidelines on compensation for medicine-induced injury.

14.10 Publication policy and press releases

Results of this trial must be submitted for publication. The Sponsor must be involved in reviewing all drafts of the manuscripts, abstracts, press releases, presentations and any other publications. Manuscripts must be submitted to the Sponsor at least 45 days in advance of being submitted for publication to allow time for the Sponsor to schedule a review and resolve any outstanding issues. Abstracts, press releases and presentations must be submitted to the Sponsor at least 14 days in advance of being released.

The CI should be the principal author and any Investigator recruiting ≥10% of patients should be listed as an author in order of numbers of patients recruited.

Authors must acknowledge that the trial was sponsored by and performed with the support of the Sponsor.

The contribution of the Sponsor should be recognised by at least one member of staff being included as an author on any publication. The BDU have manufactured AST-VAC2 and so at least one member of the BDU staff should also be included as an author.

14.11 Guidance for disruption to trial conduct

In the event of disruption to trial activities, as demonstrated during the COVID-19 pandemic, the Sponsor 'Trial Disruption' policy should be reviewed to flag trial activities which the study team should consider for adaptation during the event. A risk-based approach will be applied to each trial to inform trial conduct throughout the period in order to protect the health and well- being of existing and future trial participants and ensure compliance with current regulatory guidance.

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16 APPENDICES

16.1 APPENDIX 1: WHO PERFORMANCE STATUS SCALE

Activity Performance Description	Score
Fully active, able to carry out all normal activity without restriction.	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, for example, light housework, office work.	1
Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	2
Capable of only limited self-care. Confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair.	4

16.2 APPENDIX 2: NEW YORK HEART ASSOCIATION (NYHA) SCALE

Class I – Patients with cardiac disease but without resulting limitation of physical activity; ordinary physical activity does not cause undue dyspnoea (or fatigue, palpitation or anginal pain).

Class II – Patients with cardiac disease resulting in slight limitation of physical activity; they are comfortable at rest; ordinary physical activity results in dyspnoea (or fatigue, palpitation or anginal pain).

Class III – Patients with cardiac disease resulting in marked limitations of physical activity; they are comfortable at rest; less than ordinary physical activity causes dyspnoea (or fatigue, palpitation or anginal pain).

Class IV – Patients with cardiac disease resulting in inability to carry out physical activity without discomfort; symptoms of dyspnoea (or of angina) may be present even at rest; if any physical activity is undertaken, discomfort is increased.

16.3 APPENDIX 3: MEASUREMENT OF DISEASE

Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumours: Immune-Related Response Criteria (irRC)

Wolchok J. et al. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumours: Immune-Related Response Criteria. Clin Cancer Res 2009;15(23):7412–20.

Introduction

Investigators have relied on Response Evaluation Criteria in Solid Tumours (RECIST) or modified WHO criteria to evaluate anti-tumour responses to chemotherapeutic agents. However, the responses that are seen with immunotherapeutic agents may extend beyond those of cytotoxic agents and could include responses after disease progression that are not captured by Response Evaluation Criteria in Solid Tumours or WHO criteria. The current report presents novel criteria, designated immune-related response criteria (irRC), which can better capture the response patterns observed with some immunotherapeutic agents.

Novel to the irRC is the measurement of overall tumour burden as a metric of disease progression, compared to the limitation to baseline lesion measurements taken with WHO and RECIST. According to irRC, new lesions do not constitute disease progression if net tumour burden (including new lesions) is stable or decreases. The irRC also permit disease progression prior to response and introduce the concept of confirmation of progression at a subsequent time point after first detection. This accounts for the period required for activated T-cells to infiltrate the tumour, which may cause initial tumour volume increase but can subsequently translate into tumour shrinkage. The irRC also classify durable stable disease as clinical activity.

Immune-related response criteria

To systematically characterize additional patterns of response in patients with advanced cancer treated by immunotherapy, underlying WHO criteria were evolved into immune-related response criteria. The definitions of the irRC and guidelines on how they can be used in clinical practice are detailed below.

Antitumour response based on total measurable tumour burden

For the irRC, only index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumour burden). At the baseline tumour assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumour assessment, the SPD of the index lesions and of new, measurable lesions (≥5 × 5 mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumour burden:

$$\text{Tumour Burden} + \text{SPD}_{\text{index lesions}} + \text{SPD}_{\text{new, measurable lesions}}$$

A comparison of the use of SPD in WHO criteria versus the use of tumour burden in irRC is presented in Table 1.

Table 1. Comparison between WHO criteria and the irRC

	WHO	irRC
New, measurable lesions (i.e., ≥5 × 5 mm)	Always represent PD	Incorporated into tumour burden
New, non-measurable lesions (i.e., <5 × 5 mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumour burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumour burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumour burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

Time-point response assessment using irRC

Percentage changes in tumour burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumour assessment, the response in index and new, measurable lesions is defined based on the change in tumour burden (after ruling out irPD). Decreases in tumour burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRC were derived from WHO criteria and, therefore, the thresholds of response remain the same (Table 2).However, the irRC response categories have been modified from those of WHO criteria as detailed in Tables 1 and 2.

Table 2. Derivation of irRC overall responses

Measurable Response	Non-Measurable Response		Overall Response using irRC
Index and new measurable lesions (total measurable tumour burden)*	Non-index lesions	New, non – measurable lesions	
100% decrease	Absent	Absent	irCR†
100% decrease	Stable	Any	irPR†
100% decrease	Unequivocal progression	Any	irPR†
≥50%decrease	Absent/Stable	Any	irPR†
≥50%decrease	Unequivocal progression	Any	irPR†
<50 decrease to < 25% increase	Absent/Stable	Any	irSD
<50 decrease to < 25% increase	Unequivocal progression	Any	irSD
≥25 increase	Any	Any	irPD†

Index and non-index lesions are selected at baseline. Index lesions are measurable (≥5 x 5mm) and non-index lesions are non-measurable (<5 x 5mm, ascites, bone lesions, pleural/pericardial effusion etc.). *Changes are assessed relative to baseline and include measurable lesions only (> 5 x 5 mm)

† Assuming response and progression are confirmed by a second, consecutive assessment at least 4 weeks apart.

Overall response using irRC

The overall response according to the irRC is derived from time-point response assessments (based on tumour burden) as follows:

irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented

irPR, decrease in tumour burden ≥50% relative to baseline confirmed by a consecutive assessment at least 4 wk after first documentation

irSD, not meeting criteria for irCR or irPR, in absence of irPD

irPD, increase in tumour burden ≥25% relative to nadir (minimum recorded tumour burden) confirmation by a repeat, consecutive assessment no less than 4 wk from the date first documented

Patients were considered to have irPR or irSD even if new lesions were present, as long as they met the respective thresholds of response as described above. Furthermore, patients were not considered to have irPD if new lesions were present and the tumour burden of all lesions did not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by WHO criteria as well as those patients that shift to these irRC categories from WHO PD. Patients with irSD, particularly those with slow-declining tumour burden ≥25% from baseline at the last tumour assessment, are considered clinically meaningful because they show an objectively measurable reduction in tumour burden without reaching the 50% threshold that defines irPR (it represented an objectively measured reduction not

commonly observed in the natural history of advanced melanoma patients).

If a patient is classified as having irPD at a post-baseline tumour assessment, then confirmation of irPD by a second scan in the absence of rapid clinical deterioration is required. The definition of confirmation of progression represents an increase in tumour burden ≥25% compared with the nadir at two consecutive time points at least 4 wk apart. It is recommended that this be done at the discretion of the investigator because follow-up with observation alone may not be appropriate for patients with a rapid decline in performance status. Confirmation of irPD allows for the capture of all observed responses using the irRC (Table 2), as most of these late-responding patients have a trend toward response within 4 wk after initial irPD. Whereas WHO criteria consider any new measurable lesion to indicate PD, determination of immune-related best overall response (irBOR) is based on changes in total tumour burden from the baseline (nadir, for irPD) tumour assessment, regardless of any initial increase in baseline lesions or the appearance of new lesions.