Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
Rue de l'Institut, 89
1330 Rixensart, Belgium

Primary Study product and number	Recombinant His/NY-ESO-1 protein combined with the AS15 immunological Adjuvant System (GSK2241658A)
eTrack study number and Abbreviated Title	112406 (NYESO1-AS15-MEL-001 (MET))
Investigational New Drug (IND) number	14313
EudraCT number	2010-020663-20
Date of protocol	Final - 22 February 2010
Date of protocol amendment/administrative change	Amendment 1 Final: 18 April 2011 Administrative Change 1 Final: 17 January 2013 Amendment 2 Final: 27 November 2013 Amendment 3 Final: 03 September 2014
Title	Study of GSK2241658A Antigen-Specific Cancer Immunotherapeutic in patients with unresectable and progressive metastatic cutaneous melanoma
Detailed Title	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma
Co-ordinating author	PPD, MSc, Scientific writer
Contributing authors	PPD, MD, (***Vice President, Head of Immunotherapeutics Cancer Incubator***) PPD, MD, (*Director, Early Clinical Development, Cancer Immunotherapeutics*) PPD, MSc, (***Study Delivery Lead***, *Clinical Operations, Cancer Immunotherapeutics)* PPD, PhD, (*Clinical Scientist, Early Clinical Development, Cancer Immunotherapeutics*) PPD, (*Safety Scientist, Clinical Safety and Pharmacovigilance)* PPD, MD, (*Senior Manager, Clinical Safety and Pharmacovigilance*) PPD, (*Biostatistician, Clinical Operations*) PPD, PhD, (*Clinical Data Management, Cancer Immunotherapeutics*)

(Amended 03 September 2014)

eTrack study number and Abbreviated Title	112406 (NYESO1-AS15-MEL-001 (MET))
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Contributing authors	PPD, PhD, (Director, Head of R&D, Cancer Immunotherapeutics) PPD, (*Clinical Data Management, Cancer Immunotherapeutics*)

GSK Biologicals' Immunotherapeutic Protocol Template v 13.0 based on GSK Biologicals Protocol DS v 13.0

Protocol Amendment 3 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	112406 (NYESO1-AS15-MEL-001 (MET))
IND number	14313
EudraCT number	2010-020663-20
Date of protocol amendment	Amendment 3 Final: 03 September 2014
Detailed Title	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma
Sponsor signatory	**Frédéric Lehmann, MD** ***Vice President, Head of Immunotherapeutics Cancer Incubator (Amended 03 September 2014)***
Signature	_____
Date	_____

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Protocol Amendment 3 Rationale

Amendment number: Amendment 3

Rationale/background for changes:

The enrolment of new patients in the study, the follow-up of the patients who discontinued the study treatment due to disease progression or due to other reasons, or patients in the 1 year follow-up following the completion of the study treatment will be stopped given the fact that:

- the detailed analysis of the MAGRIT study (A double-blind, randomized, placebo-controlled Phase III study to assess the efficacy of recMAGE-A3 + AS15 Antigen-Specific Cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive Non-Small Cell Lung Cancer) showed the absence of treatment effect in any of the primary, secondary, or exploratory analyses[1]. All aspects of the MAGRIT study have been carefully assessed, and unfortunately these investigations failed to identify a root cause for the lack of efficacy of the MAGE-A3 ASCI in NSCLC.
- a comprehensive review of the MAGE-A3 ASCI Phase III results, together with all other available clinical and laboratory data in early clinical studies with various recombinant proteins, including NY-ESO-1, tested in different diseases and settings suggests that the anticancer activity of these ASCI, all based on the recombinant protein combined with adjuvant system AS15 technology may well be very limited.
- the balance of anticipated benefits and apparent risks associated with NY-ESO-1 + AS15 ASCI continues to be acceptable following the ongoing systematic review of safety data. Also, no additional safety data will be collected, except for patients who continue treatment.

By stopping the active follow-up, the patients will not be further exposed to unnecessary study related procedures.

Although GSK has no reasonable expectation of a population benefit from the treatment, GSK cannot exclude that some patients in the NYES01-AS15-MEL-001 study may benefit from this treatment on an individual basis. Therefore, patients in the study and who are currently still receiving the study treatment, will be offered the option to continue treatment if they wish to do so, following discussion with their treating physician.

[1] GSK. (April 2 2014). Update on phase III clinical trial of investigational MAGE-A3 antigen-specific cancer immunotherapeutic in non-small cell lung cancer [Press release]. Retrieved from http://www.gsk.com/media/press-releases/2014/update-on-phase-III-clinical-trial-of-investigational-MAGE-A3-antigen-specific-cancer-immunotherapeutic-in-non-small-cell-lung-cancer.html. The data will be presented at the European Society For Medical Oncology Annual Congress 2014.

In the best interest of patients, blood drawings which were planned for protocol research purposes (i.e. PBMCs, serum collection) will not be performed anymore. Blood drawing for safety monitoring as per protocol will continue.

By default, for each biological sample already collected in the scope of this study and not tested yet, testing will not be performed except if a scientific rationale remains relevant. In this case, testing will be done in compliance with the protocol and ICF signed by the patient.

The main changes in this Protocol Amendment are:

- Addition of a subsection in the introduction and rationale explaining the results of the Phase III trials with the MAGE-A3 ASCI
- Removal of all active follow-up visits and procedures,
- Addition of a statement regarding the decision not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant, and that no further blood samples for protocol research purposes will be collected.
- A clarification about the concentration in CpG7909 in different lots used in the study was added
- The list of AEs to be recorded as pIMDs was updated
- Additional clarifications were added to:
    - the study procedures
    - the hematology, serum chemistry and urine tests
    - the criteria for postponement of ASCI administrations
    - the evaluation of the overall response

In the main body of the protocol, changed text is indicated in ***bold italics***. Appendix D contains deleted text (marked with strikethrough) along with changed text. Throughout, the date of **ALL** deleted and/or changed text is **03 September 2014.**

Protocol Amendment 3 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline Biologicals (GSK Biologicals).
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals investigational product(s) and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the patient.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational product, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for 1 year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	112406 (NYESO1-AS15-MEL-001 (MET))
IND number	14313
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Date of protocol amendment	Amendment 3 Final: 03 September 2014
Detailed Title	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma
Investigator name	_____
Signature	_____
Date	_____
PPD PPD **(LKP) name:**	_____
Signature	_____
Date	_____

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SYNOPSIS

Detailed Title

An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma

Indication

Patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma (Stage III or Stage IV M1a; not Stage IV M1b or IV M1c)

Rationale for the study and study design

1. Need for new therapeutic approaches for metastatic melanoma

Patients with Stage III and IV M1a cutaneous malignant melanoma without visceral lesions have an unfavorable prognosis and there is currently no treatment able to extend survival.

The *NY-ESO-1* gene is not expressed in normal adult tissues except testis, and is expressed during embryogenesis as well as in spermatogonia and placenta as an intracellular protein. It should be noted that these normal adult cells expressing NY-ESO-1 protein do not bear Human Leukocyte Antigen (HLA) molecules on their surface and therefore do not present any NY-ESO-1-derived peptides to T-cells. By consequence, spontaneous and induced NY-ESO-1-specific immune response is unlikely to be associated with immune-related toxicities.

Because of this particular expression pattern, those genes are also called "Cancer-Testis (CT) genes". *NY-ESO-1* is an example of a CT gene. The *MAGE-A3* gene is another example of a CT gene.

The *NY-ESO-1* gene has been shown to be expressed (by Reverse Transcription Polymerase Chain Reaction; RT-PCR) in 20% to 40% of several tumor types, including breast cancer, lung cancer, prostate cancer, bladder cancer, esophageal cancer, and melanoma. The overall *NY-ESO-1* gene expression in melanoma patients is 29-43% when measured by RT-PCR, a similar range (24-45%) is determined using immunohistochemistry (IHC) in the total melanoma population, and 27-36% in the metastatic melanoma population.

NY-ESO-1 as proteins, peptides, viral vectors or plasmid DNA have been combined with a variety of immunological adjuvants in 29 clinical studies involving cancer patients with e.g. melanoma, breast, esophageal, bladder and ovarian cancers. In the large majority of these studies, a NY-ESO-1-specific $CD4^+$ and $CD8^+$ T-cell immune response has been induced, and in some patients a clinical response has been observed without major safety signal.

In a Phase II trial, recMAGE-A3 + AS15 ASCI (Antigen-Specific Cancer Immunotherapeutic) was administered to melanoma patients with early metastatic disease (GSK Study 249553/008). Objective clinical response was observed for 4 of the 36 patients, which met the criteria for proof-of-concept.

The Final Container (per human dose) is composed of 420 μg of CpG7909 oligonucleotide ***(see note below),*** which will be co-lyophilized with the 300 μg recNY-ESO-1 protein and the liquid AS01B-4 adjuvant, which contains 50 μg of QS21 (a saponin molecule) and 50 μg of Monophosphoryl lipid A (*MPL*®), in a liposome formulation.

[Note: The concentration in CpG7909 depends on the lot used – Certain lots are labeled as containing 420 μg CpG, whereas other lots will be labeled as 380 μg CpG. This difference in how the CpG content is noted is due to the fact that a different analytical method to determine the content of the CpG was applied to more recent lots and the change in the content designation from 420 to 380 μg is a result of a recalculation. The actual content of the CpG in the lots is the same (within the allowable assay variation).] (Amended 03 September 2014).

In this proposed study, recNY-ESO-1 + AS15 ASCI will be tested at the same dose schedule, in the same patient population and with the same Adjuvant System (AS15), as recMAGE A3 + AS15 ASCI in the GSK 249553/008 Study.

2. Rationale for using recNY-ESO-1 protein combined with AS15 Adjuvant System

There are two advantages in immunizing patients using the recNY-ESO-1 protein instead of peptides. First, a full protein carries multiple potential epitopes therefore targeting a broader patient population.

Second, the recNY-ESO-1 protein is also expected to carry epitopes specific for $CD4^+$ T-helper lymphocytes of the Th1 type, whose activation could significantly enhance the strength of the anti-tumor immune response.

The selection of the AS15 Adjuvant System is based on the expected benefits from a combination of immunological adjuvants, a favorable safety profile in toxicity studies evaluating the safety profile of AS15 and its individual components, and improved immunogenicity because AS15 is a strong immunostimulant in mice, monkeys and humans, and is able to induce a more powerful Th1 response and subsequently to better protect against tumor challenge than AS02B Adjuvant System.

GSK recently reported the negative results of the MAGRIT study. The detailed analysis of MAGRIT showed the absence of treatment effect in any of the primary, secondary, or exploratory analyses. All aspects of the MAGRIT study have been carefully assessed, and unfortunately these investigations failed to identify a root cause for the lack of efficacy of the MAGE-A3 ASCI in NSCLC.

Furthermore, a comprehensive review of the MAGE-A3 ASCI Phase III results, together with all other available clinical and laboratory data in early clinical studies with various recombinant proteins, including NY-ESO-1, tested in different diseases and settings suggests that the anticancer activity of these ASCI, all based on the recombinant protein combined with adjuvant system AS15 technology may well be very limited.

In light of this, GSK has decided to stop further development of the recombinant protein adjuvanted portfolio as a standalone treatment for cancer patients (Amended 03 September 2014).

3. NY-ESO-1 dose and schedule of administration

GSK will evaluate the safety and immunogenicity of recNY-ESO-1 + AS15 ASCI at a fixed dose of AS15 and antigen. GSK Biologicals will capitalize on the previous MAGE-A3 experience in melanoma through using the same dose of the tumor antigen (300 μg), the same schedule of immunization, in the same patient population and with the same AS15 Adjuvant System as the ones used for the GSK 249553/008 Study. The recMAGE-A3 + AS15 ASCI has demonstrated clinical activity in this metastatic melanoma population and this study will enable a further validation of the MAGE-A3 predictive gene signature and provide the potential to define a specific NY-ESO-1 predictive gene signature.

Objectives

Co-primary: The two co-primary objectives of this study are to document and to characterize the severe toxicity and clinical activity of the recNY-ESO-1 + AS15 ASCI in patients with NY-ESO-1-positive metastatic cutaneous melanoma.

Secondary: The secondary objectives of this study are to document and characterize:

1. Additional clinical indicators of clinical activity in the overall population and in the population of patients who present the predictive MAGE-A3 gene signature.
2. Additional indicators of safety.
3. The specific humoral and cellular immune response induced by recNY-ESO-1 + AS15 ASCI.

Translational: The exploratory objectives of this study are to document and characterize:

1. Expression testing for other tumor antigens (such as MAGE-A3, MAGE-C2, LAGE-1, PRAME and WT1) on the presenting lesion(s) and on any new lesion(s) and NY-ESO-1 expression testing on any new lesion(s).
2. The gene profiling on the tumor resected at baseline and any relapsed tumor (if available).
3. The "antigen spreading" in both the humoral and cellular immune responses elicited by the recNY-ESO-1 + AS15 ASCI.
4. The gene profiling of the immunological response elicited by the recNY-ESO-1 + AS15 ASCI.
5. Proteomics profiling of the immunological response elicited by the recNY-ESO-1 + AS15 ASCI.
6. Tumor immune infiltration assessment and characterization.
7. DNA promoter methylation analysis of NY-ESO-1, other tumor antigens and candidate genes that might be predictive to NY-ESO-1 ASCI clinical efficacy, in addition to immunoregulatory genes.
8. Pharmacogenetics analyses.

These different exploratory tests will only be performed on biological samples from patients who voluntarily gave their consent for these specific assessments in the Informed Consent Form.

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected. (Amended 03 September 2014)

Study design

- Experimental design: Single-arm, Phase I multicenter study.
- Treatment allocation: Sequential.
- Blinding: No blinding, open-label.

  Treatment Groups: Single treatment study recNY-ESO-1 + AS15 ASCI).
- Treatment administration schedule(s): patients may receive up to 4 cycles of ASCI administration (maximum of 24 ASCI administrations), provided that at each tumor evaluation time point, the clinical criteria to continue the treatment are met, including patients having a clinical response of either CR, PR, SD, or SPD.

Cycle 1: 6 ASCI administrations given at 2-week intervals (Weeks 0, 2, 4, 6, 8, 10)

Cycle 2: 6 ASCI administrations given at 3-week intervals (Weeks 14, 17, 20, 23, 26, 29)

Cycle 3: 4 ASCI administrations given at 6-week intervals (Weeks 33, 39, 45, 51)

Cycle 4: 4 ASCI administrations given at 3-month intervals followed by 4 ASCI administrations given at 6-month intervals

- Control: None.
- Type of study: Self-contained.
- Data collection: Remote Data Entry (RDE) on electronic Case Report Forms (eCRF).
- Duration of the study: The duration of the ASCI treatment period (from first visit to the concluding visit) will not last longer than 49 months for any patient, comprising the entire period of immunization and the concluding visit. The duration of the follow-up will be one year after the concluding visit. The total duration including follow-up is approximately 5 years.

  As of Amendment 3, all active follow-up visits and procedures will be stopped. Post-study AEs/SAEs will continue to be reported as described in Section 9.3.1. (Amended 03 September 2014).

Number of patients

It is planned to recruit 34 patients with metastatic melanoma (unresectable Stage III melanoma, Stage III in-transit metastasis with (N3) or without (N2c) nodal metastasis or Stage IV M1a).

Endpoints

Co-primary

Safety endpoint

- Occurrence of severe toxicities during the study treatment phase and follow-up.

Defined according to CTCAE (Version 4.0) as follows:

1. An ASCI-related or possibly ASCI-related Grade 3 or higher toxicity. Grade 3 myalgia, arthralgia, headache, fever, rigors/chills and fatigue (including lethargy, malaise and asthenia) should persist for 48 hours despite therapy in order to be taken into account.
2. An ASCI-related or possibly ASCI-related Grade 2 or higher allergic reaction occurring within 24 hours following the ASCI administration.

Note: Any severe toxicity event must be entered in the patient's eCRF within 24 hours of the event becoming known to the investigator.

Clinical activity endpoint

- The induction of objective clinical response (Complete Response (CR) or Partial Response (PR)) in the overall population.

Secondary

Clinical activity endpoints

The secondary clinical activity endpoints will include:

1. Occurrence of objective clinical response (CR or PR) in the population of patients who present the predictive MAGE-A3 gene signature.
2. In the overall population and in the population of patients presenting the predictive MAGE-A3 gene signature:
    - Occurrence of stable disease (SD).
    - Occurrence of mixed response (MR).
    - Time to Treatment Failure (TTF).

TTF is defined as the time from first treatment until the date of the last treatment administration, irrespective of the reason for study treatment discontinuation.

- Progression-free survival (PFS).

PFS is defined as the time from first treatment to either the date of ***first*** disease progression ***(PD or SPD)*** or the date of death (for whatever reason), whichever comes first. Patients alive and without disease progression are censored at the date of the last visit/contact. ***(Amended 03 September 2014)***

- Overall survival (OS).

OS is defined as the time from first treatment until death. Patients alive at the time of analysis are censored at the time of the last visit/contact.

- The duration of response for patients with CR, PR or Stable Disease (SD) status.

The duration of the response is defined as time from first objective response or SD evaluation to first PD assessment ***or death***. ***(Amended 03 September 2014)***

Safety endpoints

The secondary safety endpoints will include:

- Occurrence of adverse events (AEs) and serious adverse events (SAEs) during the study treatment period and ending 30 days after the last study treatment administration.

Note: The occurrence of SAEs (possibly) related to the recNY-ESO-1 + AS15 ASCI will be captured during the entire study duration.

Immunogenicity endpoints

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected. (Amended 03 September 2014).

- Immunogenicity of the NY-ESO-1 ASCI treatment will be evaluated at different timepoints.

On the basis of:

1. The anti-NY-ESO-1 humoral antibody concentration and response. ***(Amended 03 September 2014)***
2. The anti-NY-ESO-1 cellular (T-cell) response.

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LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse Event
AJCC	American Joint Committee on Cancer
ALAT	ALanine AminoTransferase
ANA	Anti-Nuclear Antibody
APC	Antigen Presenting Cells
APTT	Activated Partial Thromboplastin Time
ASAT	ASparate AminoTransferase
ASCI	Antigen-Specific Cancer Immunotherapeutic
ATP	According-To-Protocol
βHCG	Beta Human Chorionic Gonadotropin
BCG	Bacillus Calmette-Guérin
B-RAF	B-raf murine sarcoma viral oncogene homolog B1
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CHP	Cholesterol-bearing Hydrophobized Pullulan
CK	Creatine Kinase
CMI	Cell-Mediated Immune
CpG7909	24-nucleotide immunostimulatory oligonucleotide
CR	Complete Response
CRA	Clinical Research Associate
CT	Computer Tomography
CT gene	Cancer-Testis gene
CTA	Cancer-Testis Antigen
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Lymphocyte
DERMA	A**D**juvant Immunoth**ER**apy with MAGE-A3 in melano**MA**
DNA	DeoxyriboNucleic Acid
DSMC	Data Safety Monitoring Committee
DTIC	Dacarbazine
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration, United States

FFPE	Formalin-Fixed Paraffin Embedded
γGT	Gamma-Glutamyl Transpeptidase
GCP	Good Clinical Practice
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
GMT	Geometric Mean Titer
GSK	GlaxoSmithKline
GSM	Global Study Manager
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDDM	Insulin-Dependent Diabetes Mellitus
IEC	Independent Ethics Committee
IFNα	Interferon alpha
IFN-γ	Interferon-gamma
IHC	ImmunoHistoChemistry
IL-2	Interleukin-2
i.m.	IntraMuscular
IMAC	Ion Metal Affinity Chromatography
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	IntraUterine Device
IUS	IntraUterine System
KM	Kaplan-Meier
LICR	Ludwig Institute for Cancer Research
LD	Longest Diameter
LDH	Lactate DeHydrogenase
MAGE	Melanoma AntiGEn
MAGRIT	**M**AGE-A3 as **A**djuvant Non-Small Cell Lun**G** Cance**R** **I**mmuno**T**herapy
MedDRA	Medical Dictionary for Regulatory Activities
MET	Metastatic
MHC	Major Histocompatibility Complex
MPL	MonoPhosphoryl Lipid A
MR	Mixed Response
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose

NCI	National Cancer Institute
NK	Natural Killer
NSCLC	Non-Small Cell Lung Cancer
NY-ESO-1	Cancer-Testis gene: New York-ESOphageal cancer-1
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression-Free Survival
pIMD	potential immune mediated disease
PR	Partial Response
PREDICT	**P**redictive gene signature for **RE**sponse to recMAGE-A3 in unresecte**D** metastat**I**c **C**u**T**aneous melanoma
PT	Prothrombin Time
QS21	Saponin extracted from the tree bark of *Quillaja saponaria Molina*
RDE	Remote Data Entry
RECIST	Response Evaluation Criteria In Solid Tumors
RNA	RiboNucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SBIR	Simply the Best Internet Randomization (GSK Biologicals' system for treatment allocation using the internet)
SD	Stable Disease
SDV	Source Document Verification
SOP	Standard Operating Procedure
SPD	Slow Progressive Disease
SPM	Study Procedures Manual
SRT	Safety Review Team
SSSS	Site Staff Signature Sheet
TE	Tumor Evaluation
Th1	T-helper-1
TLR9	Toll-Like Receptor 9
TNM	The TNM staging system is based on the extent of the tumor (T), spread to lymph nodes (N), and metastasis (spread to other parts of the body) (M)
TSH	Thyroid Stimulating Hormone
TTF	Time to Treatment Failure
US	Ultrasound
USA	United States of America

GLOSSARY OF TERMS

Adequate contraception:	Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly (when applicable, as mentioned in the product label) for example abstinence, combined or progestogen oral contraceptives, injectable progestogen, implants of levonorgestrel, oestrogenic vaginal ring, percutaneous contraceptive patches or intrauterine device (IUD) or intrauterine system (IUS), vasectomy with documented azoospermia of the sole male partner or double barrier method (condom or occlusive cap plus spermicidal agent). For azoospermia, 'documented' refers to the laboratory report of azoospermia, required for acceptable documentation of successful vasectomy in the patient's male partner.
Adverse event:	Any untoward medical occurrence in a patient or clinical investigation patient, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Sections 5.6.2 and 11.4 for details on criteria for evaluability).

Global Study Manager:	An individual assigned by GSK Biologicals Headquarters who is responsible for assuring the co-ordination of the operational aspects and proper conduct of a clinical study, including compliance with International Conference on Harmonization (ICH) Harmonized Tripartite Guideline for Good Clinical Practice (GCP) and GSK policies and standard operating procedures.
Investigational study product/product: (Synonym of Investigational Medicinal Product)	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Medical Monitor:	An individual medically qualified to assume the responsibilities of the sponsor (GSK Biologicals) especially in regards to the ethics, clinical safety of a study and the assessment of adverse events.
Month	One month is defined as four weeks i.e. 28 days during the treatment phase. One year is equal to 12 months.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Patient:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the product(s) or as a control.

Patient number:	A unique number identifying a patient, assigned to each patient consenting to participate in the study/as soon as they sign the first informed consent.
Protocol amendment:	ICH defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of patients, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study. NB Any change that falls under the definition of a protocol amendment (e.g. a change that affects the safety of patients, scope of the investigation, study design, or scientific integrity of the study) MUST be prepared as an amendment to the protocol.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Study procedures manual (SPM):	Manual that provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the patients.
Sub-population:	A group of patients for whom specific data are collected compared to other patients.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a patient, identified by a unique number, according to the study randomization or treatment allocation.
Treatment number:	A number identifying a treatment to a patient, according to the study randomization or treatment allocation.

1. INTRODUCTION

1.1. Background

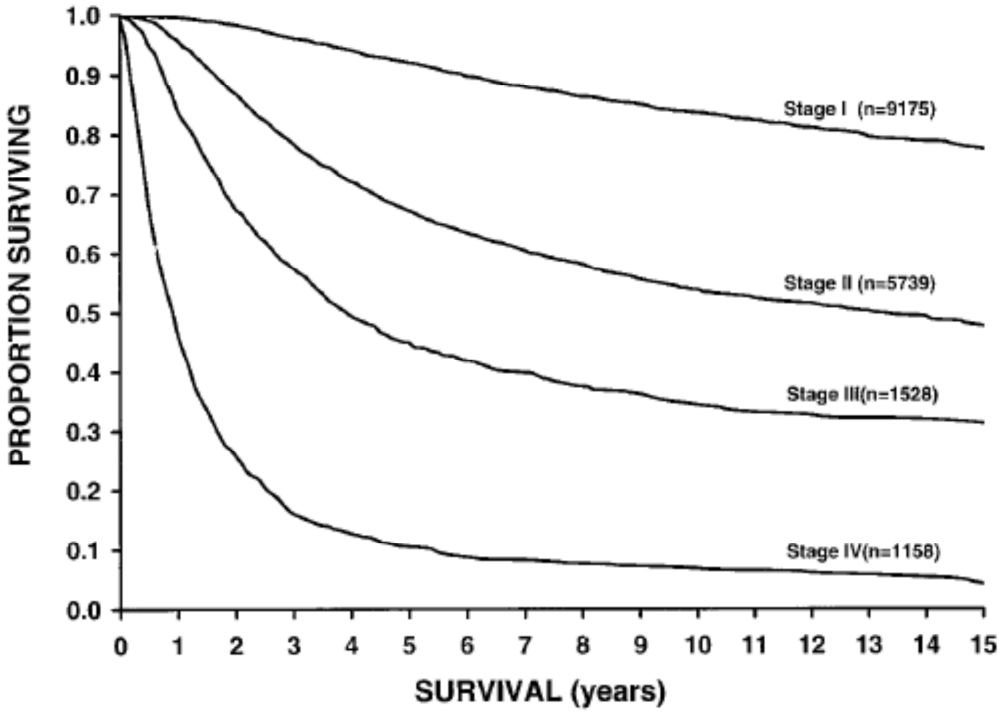
1.1.1. Background on malignant cutaneous melanoma

The incidence of malignant cutaneous melanoma is increasing rapidly having doubled in the last 10 years. The worldwide yearly incidence of melanoma is over 160,000 cases [Ferlay, 2004]. Currently, over 90% of the patients present with only primary melanoma at first diagnosis [Garbe, 2001].

Although resection of the primary tumor is curative in many cases, some 20 - 30% of the patients will eventually die of their malignant melanoma (Figure 1). Patients with distant metastases, Stage IV disease, have a very poor prognosis with a median survival of 8 months and a 2-year survival rate of 11% [Balch, 2001].

The currently most used (and to date, the only cytotoxic approved for this indication by the United States Food and Drug Administration (US-FDA)) chemotherapy in the treatment of metastatic melanoma is the tetrazine alkylating agent dacarbazine (DTIC). The response rates for single-agent dacarbazine are 10 - 20% with complete remission being rare and a median length of remission of approximately 6 months [Khayat, 2002]. Several other single-agent chemotherapies lead to similar response rates but none results in better overall survival. Combination chemotherapies with 2 - 5 drug regimens may increase response rates up to 40% but do not result in a survival advantage [Chapman, 1999]. High dose interleukin-2 (IL-2) has been licensed for use in metastatic melanoma following Phase II studies in which approximately 5% of the patients achieved long-term remissions. This regimen is, however, associated with significant toxicity and is not widely applicable [Tarhini, 2007]. Combination bio-chemotherapy interleukin-2 (IL-2) and interferon-alpha (INFα) has in several Phase II studies been demonstrated to have high response rates of 20 - 50% with a small proportion, 5 - 10%, of long-term survivors [Legha, 1998]. Disappointingly, however, Phase III studies have shown that bio-chemotherapy does not result in a survival advantage over chemotherapy in advanced melanoma [Keilholz, 2005]. Targeted therapies are also evaluated in the treatment of advanced metastatic melanoma patients. Since B-RAF (B-raf murine sarcoma viral oncogene homolog B1) has been found to be mutated in approximately 50% of melanoma patients, B-RAF inhibitors were investigated in advanced melanoma. Whereas sorafenib, a tyrosine kinase inhibitor targeting VEGFR, B-RAF and other tyrosine kinases, in combination with carboplatin and paclitaxel did not provide any advantage over placebo treatment in Phase III [Hauschild, 2009], a mutant B-RAF V600E specific inhibitor showed promising results in early clinical development [Hersey, 2009]. In a Phase II clinical study in patients with metastatic melanoma, bevacizumab, a monoclonal antibody targeting VEGF and inhibiting angiogenesis, demonstrated clinical benefit in combination with carboplatin and paclitaxel [O'Day, 2009; Perez, 2009].

Figure 1 Estimated survival curves for melanoma patients according to disease stage



Survival curves over a follow-up period of 15 years comparing localized melanoma (Stages I and II), regional metastases (Stage III), and distant metastases (Stage IV). The numbers in parentheses are patients from the AJCC melanoma staging database used to calculate the survival rates. The differences between the curves are significant ($P < 0.0001$). Adapted from [Balch, 2001].

1.1.2. Background on NY-ESO-1

The *NY-ESO-1* gene is not expressed in normal adult tissues except testis [Scanlan, 2004]. The *NY-ESO-1* gene is expressed during embryogenesis as well as in spermatogonia and placenta [Jungbluth, 2007] as an intracellular protein. It is expressed primarily in the cytoplasm although nuclear expression can be seen in some spermatogonia [Jungbluth, 2001]. It should be noted that these normal adult cells expressing NY-ESO-1 protein do not bear Human Leukocyte Antigen (HLA) molecules on their surface and therefore do not present any NY-ESO-1-derived peptides to the T-cells [Jungbluth, 2007; Guillaudeux, 1996; Simpson, 2005]. By consequence, spontaneous and induced NY-ESO-1-specific immune response is unlikely to be associated with immune-related toxicities.

Because of this particular expression pattern, those genes are also called “Cancer-Testis (CT) genes”. *NY-ESO-1* is an example of a CT gene. The *MAGE-A3* gene is another example of a CT gene [Gnjatic, 2006].

The *NY-ESO-1* gene has been shown to be expressed (by Reverse Transcription Polymerase Chain Reaction; RT-PCR) in 20% to 40% of several tumor types, including breast cancer, lung cancer, prostate cancer, bladder cancer, esophageal cancer and melanoma. The overall *NY-ESO-1* gene expression in melanoma patients is 29-43% when measured by RT-PCR [Lethé, 1998; Sahin, 1998; Sahin, 2000], a similar range (24-45%) is determined using immunohistochemistry (IHC) in the total melanoma population [Jungbluth, 2001; Prasad, 2004; Vaughan, 2004], and 27-36% in the metastatic melanoma population [Jungbluth, 2001; Prasad, 2004].

NY-ESO-1 is a very immunogenic antigen as shown by its capacity to elicit spontaneous antibody and T-cell responses in a proportion of cancer patients. Approximately 5-10% of the melanoma, ovarian or breast cancer patients and 10% of operable Non-Small Cell Lung Cancer (NSCLC) patients appear to spontaneously develop NY-ESO-1 antibodies [Stockert, 1998; Scanlan, 2002; Chapman, 2007]. The presence of spontaneous $CD4^+$ and $CD8^+$ T-cells specific for NY-ESO-1 and restricted to different HLA, have also been found in the blood of some cancer patients.

Therefore, NY-ESO-1 is a good candidate for specific immune recognition of cancer due to its restricted pattern of expression in normal tissues, but frequent occurrence in various cancers.

Please refer to the current Investigator Brochure for a review of the pre-clinical and clinical studies of the recNY-ESO-1 + AS15 ASCI.

1.2. The recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic

GSK Biologicals' candidate recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic (ASCI) comprises the recombinant His/NY-ESO-1 antigen (abbreviated as recNY-ESO-1 in the rest of this document) in combination with GSK Biologicals' proprietary immunostimulant Adjuvant System AS15.

1.2.1. The recNY-ESO-1 antigen

The recombinant NY-ESO-1-derived fusion protein (192-aa long) contains the full-length NY-ESO-1 sequence (amino acids 13-192) preceded by a 12-aa stretch comprising:

- an N-terminal tetra peptide containing the translator initiator Met: MRGS.
- a hexahistidine tag enabling the purification of the protein.
- a dipeptide linker: GS.

The fusion protein is produced in *E.coli* by employing the DNA recombinant technology. One human dose of the candidate recNY-ESO-1 + AS15 ASCI contains 300 µg of His/NY-ESO-1 antigen.

For details of this recombinant protein, see the Investigator's Brochure.

1.2.2. The AS15 Adjuvant System

The AS15 Adjuvant System consists of the Adjuvant System AS01B-4 and the immunostimulatory nucleotide CpG7909 (which is co-lyophilized with the His/NY-ESO-1 protein).

Per 0.5 mL dose, the liquid Adjuvant System AS01B-4 contains 50 µg of QS21 and 50 µg of Monophosphoryl lipid A (*MPL*®), in a liposome formulation. The immunostimulatory nucleotide CpG7909 is included ***[***420 µg per dose ***(see note in Section 5.1)]*** to enhance the immune response ***(Amended 03 September 2014)***.

For details on this Adjuvant System, please see the Investigator's Brochure.

1.2.3. Pre-clinical safety and toxicology tests with NY-ESO-1 + AS15 ASCI

The expression of the *NY-ESO-1* gene in humans is restricted only to tissues characterized by their inability to present any sort of antigens to specialized cells of the immune system, thus suggesting a lack of toxicity for humans immunized with the NY-ESO-1 protein.

No homolog close to the human *NY-ESO-1* gene could be found by mining available genomic sequences from mouse, rat, and rabbit species. Immune reactions elicited through hyper-immunization of animals might be irrelevant and of no extrapolative value to the assessment of risk to humans. Finally, no significant related or possible-related adverse events have been reported in over 3550 recorded immunizations in over 600 patients in 29 non-GSK clinical trials involving either NY-ESO-1 protein, peptides, viral vectors or plasmid DNA. The results of these trials indicated that this therapeutic approach in humans is a safe one.

Please refer to the current Investigator's Brochure for a review of the pre-clinical and clinical studies, and the potential risks and benefits of the recNY-ESO-1 + AS15 ASCI.

1.2.4. Previous clinical studies with NY-ESO-1

A large number of academic clinical trials targeting the NY-ESO-1 tumor antigen have been performed using many different immunotherapy approaches including NY-ESO-1-derived peptides or non-GSK recombinant protein combined with a non-GSK immunological adjuvant, as well as recombinant DNA or live vectors.

NY-ESO-1 recombinant protein immunotherapy have been tested in trials that involve patients with the following cancers: melanoma, breast, transitional cell carcinoma of the bladder, esophageal, prostate, sarcoma, synovial sarcoma and ovarian cancer. Other studies have involved various NY-ESO-1-derived peptide immunotherapies, NY-ESO-1 viral vector immunotherapies or a NY-ESO-1 plasmid DNA immunotherapy in patients with similar types of cancer. A variety of immunological adjuvants have been used in these studies including *ISCOMATRIX*®, Cholesterol-bearing Hydrophobized Pullulan (CHP), Bacillus Calmette-Guérin (BCG), Granulocyte Macrophage Colony-Stimulating

Factor (GM-CSF), CpG7909, *Montanide ISA®-51*, polyarginine, and the topical application of imiquimod.

No significant related or possibly-related adverse events has been reported in over 3500 recorded immunizations in over 600 patients all within the context of an anti-NY-ESO-1 immune response induced with different immunotherapy approaches and using several administration schedules and different immunological adjuvants. In the large majority of studies where immunological data is available, NY-ESO-1-specific antibodies have been detected together with a NY-ESO-1-specific $CD4^+$ and $CD8^+$ T-cell response. Some of these immunological responses have been associated with clinical activity including objective responses or long lasting disease stabilization in distinct cancer diseases.

Interestingly, in a recently reported case, a complete clinical response has been obtained in a patient with recurrent metastatic melanoma [Hunder, 2008] by adoptive transfer of *in vitro* amplified autologous $CD4^+$ T cells (3.3×10^9) specific for the DPB1*0401 restricted epitope of NY-ESO-1. The patient remained disease-free for more than 2 years with no major safety concern.

The immunological adjuvant AS15 has been administered in combination with other antigens (MAGE-A3 in NSCLC and melanoma patients, dHER2 in breast cancer patients, P501 in prostate cancer patients), showing that it is well tolerated even in the context of long-term treatment and even in older patients.

For more details on previous clinical trials targeting NY-ESO-1 tumor antigen and clinical studies with AS15 combined with other tumor antigens e.g. dHER2, P501 and recMAGE-A3 recombinant proteins, refer to the Investigator's Brochure.

1.3. Rationale for the study and study design

1.3.1. Need for new therapeutic approaches for metastatic melanoma

Patients with Stage III or IV cutaneous malignant melanoma have an unfavorable prognosis and there is currently no treatment able to extend survival. New approaches to improve the life expectancy for these patients are thus needed. Active immunization against tumor antigens is certainly one of these approaches.

NY-ESO-1 as proteins, peptides, viral vectors or plasmid DNA have been combined with a variety of immunological adjuvants in 29 clinical studies involving cancer patients with e.g. melanoma, breast, esophageal, bladder and ovarian cancers. In the large majority of these studies, a NY-ESO-1-specific $CD4^+$ and $CD8^+$ T-cell immune response has been induced, and in some patients a clinical response has been observed without major safety signal (see Section 1.2.4). In the same approach, several clinical teams have administered MAGE-A3 antigens, another tumor antigen encoded by a CT gene, to metastatic melanoma patients, using various modalities [Boon, 2006]. Again, these early clinical trials demonstrated the possibility of inducing long-term objective responses in 15% of the patients without safety signal. More specifically, in a Phase II trial, recMAGE A3 + AS15 ASCI was administered to melanoma patients with early metastatic disease (GSK Study 249553/008). Objective clinical response was observed

for 4 of the 36 patients, which met the criteria for proof-of-concept (PoC). The ASCI treatments had an acceptable safety profile and the recMAGE-A3 + AS15 ASCI is now being tested in a Phase III trial as adjuvant therapy in patients with resected melanoma (DERMA Study). In this proposed study, recNY-ESO-1 + AS15 ASCI will be tested at the same dose schedule, in the same patient population and with the same Adjuvant System (AS15), as recMAGE-A3 + AS15 ASCI in the GSK 249553/008 study. The same dose schedule is utilized in two ongoing trials: PREDICT (Predictive gene signature for REsponse to recMAGE-A3 in unresecteD metastatIc CuTaneous melanoma) and Study 111473 MAGE3-AS15-MEL-004 (MET), that are both targeting the same patient population. The patients enrolled will have Stage III or Stage IV M1a cutaneous malignant melanoma without visceral lesions.

1.3.2. Rationale for using NY-ESO-1 recombinant protein combined with AS15 Adjuvant System

1.3.2.1. recNY-ESO-1 protein

There are two advantages in immunizing patients using the recNY-ESO-1 protein instead of peptides. First, a full protein carries multiple potential epitopes therefore allowing to target a broader patient population. The eligibility of patients can be extended to all NY-ESO-1 expressing melanoma, regardless of the HLA alleles, since it may be assumed that the NY-ESO-1 protein carries additional epitopes with binding motifs specific for other HLA molecules.

Second, the recNY-ESO-1 protein is also expected to carry epitopes specific for $CD4^+$ T-helper lymphocytes of the Th1 type, whose activation could significantly enhance the strength of the anti-tumor immune response. In this regard, $CD4^+$ melanoma infiltrating lymphocytes have been shown to recognize MHC Class II-restricted tumor epitopes on human melanoma cells [Le Drean, 1995].

1.3.2.2. AS15 Adjuvant System

The immunological Adjuvant System AS15 has been selected for clinical development of the recNY-ESO-1 + AS15 ASCI. AS15 is the same immunological Adjuvant System that is included in the GSK candidate recMAGE-A3 + AS15 ASCI, which is currently under development in the Phase III pivotal studies in NSCLC (MAGE-A3 as Adjuvant Non-Small Cell LunG CanceR ImmunoTherapy or MAGRIT) and melanoma (ADjuvant immunothERapy with MAGE-A3 in melanoMA or DERMA), based on previous positive PoC Phase II studies (please refer to the Investigator's Brochure for further details of the AS15 Adjuvant System).

This selection is based on:

- **Benefits expected from a combination of immunological adjuvants**

The AS02B adjuvant contains a Toll-like Receptor 4 (TLR4) agonist (Monophosphoryl lipid A or *MPL*®) and a purified saponin molecule (QS21) in a GSK proprietary

oil-in-water emulsion. It has been shown to induce both humoral and T-cell-mediated immune responses and to lead to the induction of a systemic anti-tumor response [Gerard, 2001; Moore, 1999]. Several preclinical and clinical data support the fact that the addition of CpG to *MPL*® and QS21 (in a liposome formulation to form the AS15 Adjuvant System) can induce stronger immune and anti-tumor responses [Krieg, 2001; Meidenbauer, 2004; Ren, 2004]. CpG is a Toll-like Receptor 9 (TLR9) agonist and therefore triggers the activation of distinct sub-populations of immune cells [Akira, 2006; Pulendran, 2004; Seya, 2006], therefore eliciting broader immune responses when combined with other immunostimulants (GSK Biologicals data and [Speiser, 2005]).

- **Favorable safety profile**

Toxicity studies evaluating the safety profile of AS15 and its individual components have been conducted and no safety concern was raised.

When recMAGE-A3 + AS15 ASCI and recMAGE-A3 + AS02B ASCI were evaluated in parallel in a melanoma trial (Phase II GSK Study 249553/008), no difference in safety was observed. In addition, the AS15 immunological adjuvant has been administered in combination with ASCI antigens other than MAGE-A3 (dHER2 in breast cancer patients and P501 in prostate cancer patients) showing that it is well tolerated in the various target populations. These previous studies with AS15 also support good tolerability in long-term treatment and in elderly patients.

- **Improved immunogenicity**

AS15 is a strong immunostimulant in mice, monkeys and humans, and is able to induce a more powerful Th1 response and subsequently to better protect against tumor challenge than AS02B ([Speiser, 2005] and unpublished GSK Biologicals data).

Moreover, in the melanoma trial (Phase II GSK Study 249553/008), the combination of recMAGE-A3 + AS15 ASCI compared to recMAGE-A3 + AS02B ASCI yielded higher specific antibody titers, increased $CD4^+$ T-cell induction and a significant benefit to overall survival of patients.

GSK recently reported the negative results of the MAGRIT study. The detailed analysis of MAGRIT showed the absence of treatment effect in any of the primary, secondary, or exploratory analyses[2]. All aspects of the MAGRIT study have been carefully assessed, and unfortunately these investigations failed to identify a root cause for the lack of efficacy of the MAGE-A3 ASCI in NSCLC.

Furthermore, a comprehensive review of the MAGE-A3 ASCI Phase III results, together with all other available clinical and laboratory data in early clinical studies with various recombinant proteins, including NY-ESO-1, tested in different diseases and settings suggests that the anticancer activity of these ASCI, all based on the recombinant protein combined with adjuvant system AS15 technology may well be very limited.

In light of this, GSK has decided to stop further development of the recombinant protein adjuvanted portfolio as a standalone treatment for cancer patients (Amended 03 September 2014).

1.3.3. NY-ESO-1 dose and schedule of administration

GSK will evaluate the safety and immunogenicity of recNY-ESO-1 + AS15 ASCI at a fixed dose of AS15 and antigen. The same dose of AS15 has been combined with the other ASCI antigens under development, including MAGE-A3, dHER-2/Neu and PRAME. The amount of the recombinant NY-ESO-1 protein will be 300 μg. The dose proposed for NY-ESO-1 (i.e. 300 μg) is within the range of or equal to antigen doses used in GSK clinical trials of dHER-2/Neu and recMAGE-A3 and is also based on two previous clinical studies using NY-ESO-1 recombinant protein combined with different immunoadjuvants [Davis, 2004; Valmori, 2007].

For details of these previous clinical trials, refer to the Investigator's Brochure.

The disease is considered aggressive, given the high rate of progression. Therefore, a sustained schedule of treatment administration is proposed for these patients. The dose of the tumor antigen (300 μg) and the schedule of immunization will be exactly the same as the ones used for the GSK 249553/008 proof of concept study with the recMAGE-A3 + AS15 ASCI in patients with metastatic cutaneous melanoma.

In the proposed Phase I study, the endpoints will not be evaluated according to a dose escalation study design. It is acknowledged that the requirement to determine the maximum tolerated dose (MTD) is based on the design of conventional Phase I dose-escalation trials used for cytotoxic drugs. It is supported by the assumption that

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[2] GSK. (April 2 2014). Update on phase III clinical trial of investigational MAGE-A3 antigen-specific cancer immunotherapeutic in non-small cell lung cancer [Press release]. Retrieved from http://www.gsk.com/media/press-releases/2014/update-on-phase-III-clinical-trial-of-investigational-MAGE-A3-antigen-specific-cancer-immunotherapeutic-in-non-small-cell-lung-cancer.html. The data will be presented at the European Society For Medical Oncology Annual Congress 2014.

maximizing dose should maximize clinical efficacy and that the anti-tumor activity of a non-specific cytotoxic agent is inevitably connected to toxicity risks. However, antigen-specific cancer immunotherapeutics are generally believed to be safe relative to cytotoxic agents. In addition, the NY-ESO-1 CT gene is only expressed in cancer cells, spermatogonia, placenta tissue and during embryogenesis. The spermatogonia and placenta tissues expressing NY-ESO-1 protein do not bear HLA molecules on their surface and therefore do not present any NY-ESO-1-derived peptides to the T-cells [Jungbluth, 2007; Guillaudeux, 1996; Simpson, 2005]. By consequence, and considering this specific pattern of expression, spontaneous and induced NY-ESO-1-specific immune responses are unlikely to be associated with immune-related toxicities.

1.3.4. Rationale for translational research

Previous clinical studies performed with the adjuvanted recombinant MAGE-A3 protein in metastatic melanoma patients have led to encouraging sign of clinical activity, leading to long-term objective clinical responses and stable disease. In parallel, we showed that antibodies and T-cell responses specific to the MAGE-A3 antigen can be measured in the immunized patients [Kruit, 2008].

However, so far it has been difficult to establish a true correlation between one of the immune parameters as measured on peripheral blood samples from the patients and the clinical benefit to the patients. Obtaining access to tissues other than blood (tumor samples taken at different moments during or after treatment) to explore other new immune read-outs or other ways to assess the immune response using genomics or proteomics could be very informative.

In parallel to the monitoring of the immune response induced in the patients by MAGE-A3 ASCI, in a previous study the gene profile of tumors before immunization using an Affymetrix microarray platform was analyzed. This allowed the definition of a gene signature which is associated with clinical benefit (including objective response, mixed response, and long-term disease stabilization). Estimated Kaplan-Meier curves of the Time to Treatment Failure (TTF) for the recMAGE-A3 + AS15 ASCI group of patients from the GSK 249553/008 melanoma trial also suggest that the presence of the gene signature correlates with a favorable clinical outcome in response to the recMAGE-A3 + AS15 ASCI therapy [Louahed, 2008]. This set of genes differentially expressed in MAGE-A3 positive melanoma patients with clinical benefit in response to the MAGE-A3 ASCI treatment will in this protocol be referred to as the predictive MAGE-A3 gene signature. It will be interesting to evaluate if the MAGE-A3 gene signature can predict clinical benefit in patients that receive the recNY-ESO-1 + AS15 ASCI. In addition, it would also be interesting to explore if a specific predictive *NY-ESO-1* gene signature exists, that is different to the predictive MAGE-A3 gene signature.

These measurements are described in detail in Section 7, and their objectives are delineated in the corresponding sections. Each of them is essential for an understanding of the induction of an immune response, as together they allow insight into the "cross-talk" between the tumor and its environment and ultimately the regulation by the immune system of its anti-tumor response.

These different translational and additional exploratory tests will only be performed on biological samples from patients who voluntarily gave their consent for these specific assessments on the Informed Consent Form (ICF).

2. OBJECTIVES

2.1. Co-primary objectives

The two co-primary objectives of this study are to document and to characterize the severe toxicity and clinical activity of the recNY-ESO-1 + AS15 ASCI in patients with NY-ESO-1-positive metastatic cutaneous melanoma.

Refer to Section 11.1 for the definition of the primary endpoints.

2.2. Secondary objectives

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.(Amended 03 September 2014).

The secondary objectives of this study are to document and characterize:

1. Additional clinical indicators of clinical activity in the overall population and in the population of patients who present the predictive MAGE-A3 gene signature.
2. Additional indicators of safety.
3. The specific humoral and cellular immune response induced by recNY-ESO-1 + AS15 ASCI.

Refer to Section 11.2 for the definition of the secondary endpoints.

2.3. Translational research objectives

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.(Amended 03 September 2014).

The exploratory objectives of this study are to document and characterize:

1. Expression testing for other tumor antigens (such as MAGE-A3, MAGE-C2, LAGE-1, PRAME and WT1) on the presenting lesion(s) and on any new lesion(s) and NY-ESO-1 expression testing on any new lesion(s).
2. The gene profiling on the tumor resected at baseline and any relapsed tumor (if available).

3. The "antigen spreading" in both the humoral and cellular immune responses elicited by the recNY-ESO-1 + AS15 ASCI.
4. The gene profiling of the immunological response elicited by the recNY-ESO-1 + AS15 ASCI.
5. Proteomics profiling of the immunological response elicited by the recNY-ESO-1 + AS15 ASCI.
6. Tumor immune infiltration assessment and characterization.
7. DNA promoter methylation analysis of NY-ESO-1, other tumor antigens and candidate genes that might be predictive to NY-ESO-1 ASCI clinical efficacy, in addition to immunoregulatory genes.
8. Pharmacogenetics analyses.

These different exploratory tests will only be performed on biological samples from patients who voluntarily gave their consent for these specific assessments in the ICF.

3. STUDY DESIGN OVERVIEW

3.1. General outline of study design

This will be an open-label, single-group, multicenter Phase I study, planned to be conducted at approximately 20 centers.

Approximately 34 patients with metastatic melanoma (unresectable Stage III melanoma, Stage III in-transit metastasis with (N3) or without (N2c) nodal metastasis or Stage IV M1a) will be enrolled. All patients will be treated as out-patients and will receive the same treatment. Withdrawals will not be replaced in this study.

Data collection will be conducted by using Remote Data Entry (RDE) on electronic case report forms (eCRF).

Adverse events, including severe toxicity as defined in Section 11.1.1, will be monitored throughout this Phase I study.

The first five patients will be enrolled in a staggered manner controlled by a central process such that they will receive their first ASCI administration on different days. For example, one patient immunized per day until five patients have been immunized. The other patients can receive their first ASCI administration at any time starting from one day after the fifth patient has received his/her first ASCI administration.

A centralized process is in place to ensure that all the investigators are informed about any serious adverse event (SAE) considered as related or possibly related to the ASCI, i.e., when an investigator becomes aware of such an SAE in one of his patients the Sponsor should be notified immediately (within 24 hours, refer also to Section 9.4.3).

3.2. Study Treatment Schedule

Patients may receive up to four cycles of ASCI administration (maximum of 24 ASCI administrations), provided that at each tumor evaluation time point, the clinical criteria to continue the treatment are met:

Cycle 1:	6 ASCI administrations, each given at 2-week intervals (Weeks 0, 2, 4, 6, 8, 10)
Cycle 2:	6 ASCI administrations, each given at 3-week intervals (Weeks 14, 17, 20, 23, 26, 29)
Cycle 3:	4 ASCI administrations, each given at 6-week intervals (Weeks 33, 39, 45, 51)
Cycle 4:	4 ASCI administrations, each given at 3-month intervals 4 ASCI administrations, each given at 6-month intervals

The patient's clinical response to the treatment will be assessed regularly (for details, see Section 6.6), because this will determine, whether (s)he may continue receiving the study treatment. The clinical responses qualifying for a continuation of the study treatment are set out in Section 6.5.3.11.

3.3. Duration of study participation

For each patient, the maximal duration of the study treatment period will be approximately 4 years (49 months) from the first administration of the study treatment until the concluding visit (Table 1).The duration of active follow-up for survival, disease progression and serious adverse events (SAEs) related to study participation or related to concurrent use of GSK medications will be one year after the concluding visit. During the follow-up period, the patient will have to come for follow-up visits every 3 months. Patients withdrawn from study treatment because of disease progression will *not* be asked to come for such follow-up visits *but* will be followed for survival by means of bi-annual contacts (e.g., by phone or during standard visits of the patient to the institution). All patients should be followed up for survival for a minimum of 5 years after the first study treatment administration, regardless of disease progression and of when they cease to receive the study treatment. For patients having completed (part of) the follow-up visits, the further follow-up for survival up to 5 years after the first study treatment administration will be done by bi-annual contacts, e.g., by phone or during the patient's standard visits to the institution. Patients withdrawn from the study because they withdraw their consent to participate in the study will not be contacted in this way.

As of Amendment 3, all active follow-up visits and procedures will be stopped. Post-study AEs/SAEs will continue to be reported as described in Section 9.3.1. (Amended 03 September 2014.)

Table 1 Overview of ASCI administrations, tumor and immunological evaluations [1]

Cycle no.		***Cycle 1***							***Cycle 2***							
Interval between ASCI administrations (admin.)		**2 weeks**							**3 weeks** *Continue treatment if CR or PR or SD or SPD at each TE*							
Visit no.	Screening	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Weeks after first ASCI admin. (w)	w-6 to w-1	0	2	4	6	8	10	12	14	17	20	22	23	26	29	31
recNY-ESO-1 + AS15 admin no.		**1**	**2**	**3**	**4**	**5**	**6**		**7**	**8**	**9**		**10**	**11**	**12**	
Tumor evaluation (TE)	**X**							**X**				**X**				**X**
Anti-NY-ESO-1 Ab response		**X**		**X**		**X**	**X**	**X**							**X**	
Anti-NY-ESO-1 T cell responses		**X**					**X**								**X**	

Cycle no.	***Cycle 3***					***Cycle 4***								
Interval between ASCI admin.	**6 weeks**					**3 months**				**6 months**				**Concluding Visit**
	Continue treatment if CR or PR or SD or SPD at each TE													
Visit no.	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*
Week (w), month (m) or year (y) after first ASCI admin.	33w	39w	45w	51w	54w	V19 +3m	V21 +3m	V22 +3m	V23 +3m	V24 +6m	V25 +6m	V26 +6m	V27 +6m	V28 + 30 days
recNY-ESO-1 + AS15 admin no.	**13**	**14**	**15**	**16**		**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	
Tumor evaluation (TE)					**X**		**X**		**X**	**X**	**X**	**X**		**X**
Anti-NY-ESO-1 Ab response				**X**			**X**		**X**	**X**	**X**	**X**		**X**
Anti-NY-ESO-1 T cell responses				**X**			**X**		**X**	**X**	**X**	**X**		**X**

1. Abbreviations: Ab - Antibody; Admin - Administration; CR - Complete Response; m = Month (Month, as calculation convention: 1 month is defined as 4 weeks i.e. 28 days during the treatment phase); PR - Partial Response; SD - Stable Disease; SPD - Slow Progressive Disease; TE - Tumor Evaluation; w - Week; y - Year.

2. In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administration" rather than by the "weeks after first study treatment administration".

(Amended 03 September 2014)

4. STUDY POPULATION

4.1. Number of patients/centers

It is planned that recruitment will stop when 34 patients have been enrolled into the study. Patients who have entered the screening phase and signed the ICF at the time, when the targeted number of patients has been reached, will be allowed to enroll in the study.

4.1.1. Overview of the recruitment plan

The recruitment of patients will include the following essential aspects:

- Approximately 20 study centers will participate.
- Enrolment will take place by these centers in parallel and be competitive. No limit will be placed on the number of patients that can be recruited by any center.
- Recruitment is expected to start in February 2011 and is expected to be completed within approximately 1 year, i.e., by the end of the 1st quarter of 2012. If this target cannot be met, the recruitment period may be extended as required.
- Recruitment tracking will be performed centrally by GSK Biologicals. Monitoring will include supervision of adherence to the recruitment plan and will be carried out by qualified and authorized people who work for or with GSK Biologicals. A screening log will be kept at each site. All patients screened will be entered into the screening log.

4.1.2. Replacement

Patients withdrawn after having received the first dose of study treatment will not be replaced. Any patient withdrawn before receiving the first ASCI dose will be replaced.

4.2. Inclusion criteria

The following criteria should be checked during the screening procedures and again just before the first ASCI dose. The patient may only be included in the study if ALL the following inclusion criteria are fulfilled:

1. Male or female patient with histologically proven, measurable metastatic cutaneous melanoma, and with documented progressive disease within the 12 weeks before the first administration of study treatment.

According to the [American Joint Committee on Cancer], 2002 classification, all melanoma patients with measurable, unresectable Stage III melanoma, Stage III in-transit metastasis with (N3) or without (N2c) nodal metastasis and Stage IV M1a melanoma are candidates for inclusion, whereas patients with resected Stage IV and Stage IV M1b or IV M1c disease **cannot** be included in the study (for the TNM classification, see Appendix A).

2. Written informed consent for NY-ESO-1 expression screening and gene profiling on resected tumor tissue and for the complete study has been obtained from the patient prior to shipment of the sample for expression testing and prior to the performance of any other protocol-specific procedure.

Note: Procedures performed before obtaining the patient's informed consent as part of standard institution practices or in the context of another research study (see Section 6.4) are accepted as study procedures provided the time intervals stipulated in the protocol are observed.

3. Patient is ≥ 18 years of age at the time of signature of the informed consent.
4. The patient's tumor shows expression of NY-ESO-1, as determined by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis or any updated technique on fresh tissue sample(s).
5. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
6. The patient has normal organ functions as shown by all of the following:
   - Hemoglobin ≥ 12 g/dL
   - Absolute leukocytes count ≥ 3.0 x 10^9/L
   - Absolute lymphocytes count ≥ 1.0 x 10^9/L
   - Platelets ≥ 100 x 10^9/L
   - Serum creatinine ≤ Upper Limit of Normal (ULN)
   - Serum total bilirubin ≤ 1.5 x ULN (except for patients with Gilbert's syndrome for whom the limit is 2 x ULN)
   - Lactate dehydrogenase (LDH) ≤ ULN
   - Aspartate aminotransferase (ASAT) ≤ 2 x ULN
   - Alanine aminotransferase (ALAT) ≤ 2 x ULN

   These tests musts be done no more than 3 weeks before the first ASCI administration.
7. Female patients of non-childbearing potential may be enrolled in the study. Non-childbearing potential is defined as current tubal-ligation, hysterectomy, ovariectomy or post-menopause.

Please refer to the glossary of terms for the definition of menopause.

8. Female patient of childbearing potential may be enrolled in the study, if the patient:
   - has practiced adequate contraception for 30 days prior to first ASCI administration, and
   - has a negative pregnancy test at the specified study visits, and

- has agreed to continue adequate contraception during the entire treatment period and for 2 months after completion of the ASCI administration series.

Please refer to the glossary of terms for the definition of adequate contraception.

A pregnancy test must be done no more than one week before the first ASCI administration.

9. In the view of the investigator, the patient can and will comply with the requirements of this protocol.

4.3. Exclusion criteria for enrolment

The following criteria should be checked during the screening procedures and again just before the first ASCI administration. If **ANY** exclusion criterion applies, the patient must not be included in the study:

1. The patient has at any time received systemic chemotherapy, biochemotherapy, small molecules or anti-CTLA-4 monoclonal antibody for metastatic disease.

Note: Isolated limb perfusion or ***local electrochemotherapy***, as long as this was performed at least 4 weeks before first ASCI administration is authorized. ***(Amended 03 September 2014).***

2. The patient is scheduled to receive any other anticancer treatments than those specified in the protocol, including but not limited to (bio-)chemotherapeutic, immunomodulating agents and radiotherapy.
3. The patient received any cancer immunotherapy containing a NY-ESO-1 antigen or any cancer immunotherapy for his/her metastatic disease.

Note: Previous adjuvant treatment with interferon, anti-CTLA-4 monoclonal antibody or a cancer immunotherapeutic ("vaccine") containing a tumor antigen other than NY-ESO-1 ***or radiotherapy*** is allowed, if the last administration took place at least 8 weeks before the first ASCI administration. ***(Amended 03 September 2014).***

4. The patient requires concomitant treatment (more than 7 consecutive days) with systemic corticosteroids, or any other immunosuppressive agents.

Exception: The use of prednisone, or equivalent, at a dose ≤ 0.125 mg/kg/day (absolute maximum 10 mg/day) for more than 7 consecutive days or at a dose > 0.125 mg/kg/day but for less than 7 consecutive days is allowed. The use of inhaled corticosteroids or topical steroids is also permitted.

5. Use of any investigational or non-registered product (drug or vaccine) other than the ASCI within 30 days preceding the first ASCI administration, or planned use during the study period.
6. The patient has (had) previous or concomitant malignancies at other sites (including carcinoma in situ), except effectively treated non-melanoma skin cancers or carcinoma in situ of the cervix or effectively treated malignancy that has been in remission for over 5 years and is highly likely to have been cured.

The patient has an allergy to any component of the study investigational product or has a history of previous allergic reactions to vaccinations.

7. The patient has an autoimmune disease such as, but not limited to, multiple sclerosis, lupus, and inflammatory bowel disease. Patients with vitiligo are not excluded.
8. The patient has a family history of congenital or hereditary immunodeficiency.
9. The patient is known to be positive for the Human Immunodeficiency Virus (HIV).

Specification for Germany: Please, refer to Section 13.2.

10. The patient has an uncontrolled bleeding disorder.
11. The patient has psychiatric or addictive disorders that may compromise his/her ability to give informed consent, or to comply with the trial procedures.
12. The patient has concurrent severe medical problems, unrelated to the malignancy, that would significantly limit full compliance with the study or expose the patient to unacceptable risk.
13. For female patients: the patient is pregnant or lactating.

5. STUDY PRODUCT AND ADMINISTRATION

5.1. Description of study product

The recNY-ESO-1 + AS15 ASCI to be used has been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for this candidate ASCI are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis) and the required approvals have been obtained.

The ASCI is labeled and packed according to applicable regulatory requirements.

In this study, the recNY-ESO-1 + AS15 ASCI will be administered by using a sterile two-vials set comprising:

- One vial containing 300 µg of the recNY-ESO-1 protein co-lyophilized with 420 µg of CpG7909;

[Note: The concentration in CpG7909 depends on the lot used –Certain lots are labeled as containing 420 µg CpG, whereas other lots will be labeled as 380 µg CpG. This difference in how the CpG content is noted is due to the fact that a different analytical method to determine the content of the CpG was applied to more recent lots and the change in the content designation from 420 to 380 µg is a result of a recalculation. The actual content of the CpG in the lots is the same (within the allowable assay variation).] (Amended 03 September 2014).

- One vial with liquid Adjuvant System AS01B-4 (0.5 mL; liposomes containing 50 μg of monophosphoryl lipid A (*MPL*®), 1000 μg of dioleylphosphatidylcholine (DOPC), 250 μg of cholesterol and 50 μg of QS21 (QS21 is a natural saponin molecule extracted from bark of the South-American tree Quillaja *saponaria molin*) in phosphate-buffered saline, making up the remainder of the Adjuvant System AS15).

The final recNY-ESO-1 + AS15 ASCI for administration is obtained by reconstitution of the lyophilized preparation with the adjuvant diluent. A recNY-ESO-1 + AS15 ASCI dose consists of 0.5 mL.

The recNY-ESO-1 + AS15 ASCI will be administered by intramuscular injection (i.m.).

5.2. Storage and handling of study product

All ASCI products to be administered to the patients must be stored in a safe and locked place with no access by unauthorized personnel.

The ASCI product must be stored at the defined temperature range (i.e., 2 to 8°C/36°F to 46°F). Please refer to the Study Procedures Manual (SPM) for more details on storage of the ASCI. The storage temperature of the ASCI will be monitored daily with validated temperature monitoring device(s) (at the minimum calibrated) and will be recorded as specified in the SPM.

The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact.

Any temperature deviation outside the range (0-8°C/36-46°F), must be reported to the Sponsor as soon as detected. Following an exposure to such a temperature deviation, ASCI will not be used until approval has been given by the Sponsor. In case of temperature deviation between 0 and +2°C/32 and 36°F, the impacted study products can still be administered, but the site must take adequate actions to go back to the defined range +2 to +8°C/36 to 46°F and avoid re-occurrence of such a temperature deviation.

Refer to the SPM for details and instructions on the temperature deviation process, packaging and accountability of the ASCI.

5.3. Dosage and administration of study product

5.3.1. Dosage

A standard dose of recNY-ESO-1 protein (300 μg) and of AS15 will be used, irrespective of the patient's body weight or body surface area (BSA).

No modification of the dose of the study product is permitted for any individual patient, irrespective of the patient’s weight or BSA.

5.3.2. Administration

The needles used for study treatment administration should be suitable for intramuscular (i.m.) injection. The entire liquid content of the diluent vial is to be transferred aseptically into the vial containing the lyophilized preparation. The vial is to be shaken gently until complete dissolution of the pellet. The reconstituted mixture must be at a suitable temperature (i.e., no longer chilled) when it is administered. The final preparation is to be injected immediately or, if not possible, no later than 4 hours after reconstitution and provided that the final preparation has been kept at a temperature between 4°C (39°F) and 30°C (86°F).

The investigator or designate will subsequently withdraw the reconstituted mixture, change the needle, and inject the dose slowly (over approximately 30 seconds) i.m. into thigh or deltoid. The site of injections should be alternated to prevent the patient's discomfort. The injections may not be administered in anatomical regions where lymph nodes have been excised.

The patients will be observed closely for at least 30 minutes following the administration of the ASCI treatment, with appropriate medical treatment readily available in case of a rare anaphylactic reaction.

For treatment schedules, see the in-text table in Section 3.1. For exact specification of the visits and for calculation of the duration of treatment, see Section 6.

Table 2 Dosage and administration

Treatment	Dose	Administration	
		Timing	***Route and site***
recNY-ESO-1 + AS15 ASCI	1 dose corresponding to 300 μg of recNY-ESO-1 protein and ***380*** μg CpG, reconstituted in AS01B-4 ***(Amended 03 September 2014)***	Cycle 1: q2w x 6 Cycle 2: q3w x 6 Cycle 3: q6w x 4 Cycle 4: q3m x 4 then q6m x 4	i.m. Deltoid or lateral region of the thigh, preferably with alternation on right or left side at each succeeding injection

5.4. Replacement of unusable study product doses

Additional ASCI doses will be provided to replace those that are unusable (see SBIR manual for details).

The Investigator or delegate will request a new treatment number from GSK Biologicals (SBIR system) via the Internet. He/she will access the allocation system and enter the patient's ID number and date of birth. The allocation system will then determine the treatment number to be used for the immunization of that patient.

The actual treatment number used for the study treatment administration of the patient must be recorded in the eCRF (treatment allocation section).

5.5. Contraindications to subsequent study product administration

The criteria in the following subsections should be checked at each visit. If any of these events occurs during the study, this will require appropriate action, i.e. postponement or permanent stopping of the ASCI administration (See Sections 5.5.1 and 5.5.2).

5.5.1. Criteria for postponement of ASCI administration

If one of the following conditions is present at the time scheduled for administration of the recNY-ESO-1 + AS15 ASCI, the patient may be treated at a later date (i.e. the entire program of study visits and ASCI administrations is interrupted), within the time window specified in Section 6.3.1, or as soon as the patient's condition allows it, or withdrawn at the discretion of the investigator.

1. Acute disease is defined as the presence of a moderate or severe illness with or without fever. All ASCIs can be administered to persons with a minor illness such as diarrhea or mild upper respiratory infection without fever. The patient must be followed until resolution of the event, as with any adverse event (AE) (see Section 9.3).
2. Fever, defined as an oral, axillary or tympanic temperature of 38°C/100°F or above.
3. ***Any other medical reason that would expose the patient to an unacceptable risk, at the investigator's discretion (Amended 03 September 2014).***

For these ***three*** conditions, the entire program of study visits and ASCI administrations will be resumed as soon as the patient's condition allows or at the investigator's discretion (see Section 6.3.1). If the re-treatment criteria are not met within 4 weeks (3 months during and after Cycle 4) after the scheduled treatment date, the patient must be withdrawn from the study treatment and the procedures detailed for the Concluding Visit are to be carried out (see Section 6.5.4). ***(Amended 03 September 2014).***

In the framework of an imposed vaccination program, the ASCI administration may be postponed in order to administer the influenza vaccine on the fixed day. The ASCI administration should be given as close in time as possible to the time scheduled in the original administration schedule. However, a 6 day interval should be maintained between the influenza vaccine and the ASCI administration (see also Section 5.6.3 on time window for prophylactic vaccination).

5.5.2. Criteria for permanent stopping of ASCI administration

If any of the following criteria becomes applicable during the study, the patient will be required to discontinue the study ASCI administration.

1. Evidence of disease progression that does not correspond to the Slow Progressive Disease (SPD) (See Section 6.6.6 and Section 6.5.3.11 Clinical response criteria for continuing ASCI administration).
2. Treatment with one of the following:

- Investigational or non-registered product other than the study recNY-ESO-1 + AS15 ASCI.
- Other anticancer treatments, including but not limited to (bio-)chemotherapeutic or immunomodulating agents and radiotherapy.

However, any progression of some tumor sites or symptoms can be controlled by surgery or radiotherapy (≤ 20 Grays); see Section 5.6.1 for further details.

3. ASCI-related or possibly ASCI-related Grade 2 (or higher, according to Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0) allergic reaction/hypersensitivity toxicity (i.e. rash, flushing, urticaria and dyspnea). Drug fever will not be part of this definition.
4. Grade 2 (or higher, according to CTCAE, Version 4.0) autoimmune reaction (i.e. evidence of autoimmune reaction involving a non-essential organ or function such as hypothyroidism).
5. Grade 3 (or higher, according to CTCAE, Version 4.0) injection site reaction (i.e. ulceration or necrosis that is severe; operative intervention indicated).
6. Any intolerable AE, at the investigator's discretion.
7. Appearance of any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection, or any medical condition requiring chronic treatment (more than 7 consecutive days) with systemic corticosteroids or any other immunosuppressive agents.

[Note: The use of prednisone, or equivalent, at a dose ≤ 0.125 mg/kg/day (absolute maximum 10 mg/day) for more than 7 consecutive days or at a dose > 0.125 mg/kg/day but for less than 7 consecutive days is allowed. The use of inhaled corticosteroids or topical steroids is also permitted.]

8. The patient develops other conditions for which, in the investigator's opinion, the patient's best interest is to be withdrawn from the study treatment.
9. Inability of the patient to complete the study evaluations/visits because of unforeseen circumstances.
10. If in case of postponement of study treatment the re-treatment criteria are not met within 4 weeks (3 months during and after Cycle 4) after the scheduled treatment date, the patient must be withdrawn from the study treatment (see Section 5.5.1).

11. The patient requests to be withdrawn from the study treatment.
12. For female patients, pregnancy or decision to become pregnant (see Section 9.2.2).

For patients whose treatment is discontinued prematurely during the study, the procedures of the Concluding Visit (see Section 6.5.4) will be carried out a minimum of 30 days after the last ASCI administration and - if possible - before the initiation of any other treatment.***(Amended 03 September 2014).***

After disease progression, the choice of subsequent treatment will be at the discretion of the treating physician; the patient will be taken off study treatment and the investigator will document any further anti-cancer treatment administered. ***(Amended 03 September 2014)***

5.6. Concomitant medication and non-drug therapies

Patients should receive medication appropriate to their health condition during the whole study.

At each study visit/contact, the investigator should question the patient about any medication(s) taken and treatment received by the patient.

All concomitant medication, including changes in chronic medication, with the exception of vitamins and/or dietary supplements, are to be recorded in the eCRF. This also applies to any medication intended to treat an AE. Chronic medication is defined as medication taken by the patient for a minimum of 6 months before the study start.

Similarly, concomitant medication administered for the treatment of a SAE, at any time, must be recorded on the SAE screens in the eCRF, as applicable. Refer to Section 9.1.2 for the definition of a SAE.

5.6.1. Permitted medication

During the period starting with administration of each dose of study treatment and ending 30 days after each dose of study treatment, concomitant medication administered for the treatment of an AE must be recorded in the eCRF with generic name of the medication (trade names are allowed for combination drugs only), medical indication (including which AE), total daily dose, route of administration, start and end dates of treatment.

The following palliative melanoma treatments (of tumor sites or symptoms) are allowed in addition to the study treatment:

- Surgery.

Note: If surgery is performed, sample(s) of the resected tumor lesion(s) will be sent to GSK Biologicals or a laboratory contracted for this by GSK Biologicals (see Sections 7.1 and 7.2.2). For patients who have given their specific informed consent to the optional translational research, such tumor tissue(s) may also be used to conduct this specific testing (see Sections 7.3, 7.6, 7.7, and 7.8).

- Radiotherapy.

However, these treatments may not be applied to more than 20% of the number of all target lesions and must always be discussed in advance with, and agreed to, by the Sponsor. The applied radiotherapy dose must be ≤ 20 Grays.

The patients who are presenting a progression by (adapted) RECIST (Response Evaluation Criteria In Solid Tumors, see Sections 6.6.1, 6.6.2, and 6.6.3) and are receiving such treatments will be considered in Progressive Disease (PD) for the purpose of response evaluation.

5.6.2. Medications, vaccines and non-drug therapies that may lead to the elimination of a patient from ATP analyses

5.6.2.1. Prohibited medications or non-drug therapies

Patients may not receive concomitant treatment during the study with any of the following:

a. Other anticancer treatments, including but not exclusively, chemotherapeutic, immunomodulating agents and radiotherapy.

However, any progression of some tumor sites or symptoms can be controlled by surgery or radiotherapy (≤ 20 Grays); see Section 5.6.1 for further details.

b. Administration of immunosuppressants or other immune-modifying drugs during the study period. The use of prednisone, or equivalent, at a dose ≤ 0.125 mg/kg/day (absolute maximum 10 mg/day) for more than 7 consecutive days or at a dose > 0.125 mg/kg/day but for less than 7 consecutive days is allowed. The use of inhaled corticosteroids or topical steroids is also permitted.

c. Administration of any commercial anti-infectious vaccine not foreseen by the study protocol during the period within the prohibited time as specified in Section 5.6.3.

d. Administration of immunoglobulins and/or any blood products during the study period.

Any of these specifically contraindicated treatments and/or medications administered at any time during the study period are to be recorded in the eCRF with generic name of the medication (trade names are allowed for combination drugs only), medical indication, total daily dose, route of administration, start and end dates of treatment.

If any of these is taken, it will require either permanent discontinuation of study treatment (see Section 5.5.2) or may determine a patient evaluability in the according to protocol (ATP) analysis (see Section 5.6.2.2).

5.6.2.2. Elimination criteria for analysis

The following criteria should be checked at each visit subsequent to the first ASCI administration. If any become applicable during the study, it will not require withdrawal of the patient from the study but may determine a patient's evaluability in the according-to-protocol (ATP) analysis. See Section 11.4 for definition of study cohorts to be evaluated.

- Use of any investigational or non-registered product (drug or vaccine) other than the ASCI during the study period. This includes other anticancer medications e.g. chemotherapeutic agents, immunomodulating agents and radiotherapy (See Section 5.6.1).
- Administration of more than 7 days of immunosuppressants or other immune-modifying drugs during the study period. For corticosteroids, this will mean prednisone > 0.125 mg/kg/day (maximum 10 mg/day) or equivalent. Inhaled and topical steroids are allowed.
- Any progression of some tumor sites or symptoms which is controlled by surgery or by local radiotherapy. If this conventional treatment (surgery or local radiotherapy) is applied to > 20% of the total target lesions, this will not require patient withdrawal but may affect the patient's evaluability in the according to protocol (ATP) analysis.
- Administration of a product not foreseen by the study protocol during the time period specified in Section 5.6.3.
- Administration of immunoglobulins and/or any blood products during the study period.

A detailed, comprehensive list of reasons for elimination from ATP analyses will be established at the time of data cleaning.

5.6.3. Time window for concomitant vaccination

Immunization with any commercial anti-infectious vaccine may be performed during the study. However, this may not take place during the period from six days before any ASCI administration to six days after. Thus, if the ASCI is to be administered on a notional Day 0, then the vaccination may be performed on or before Day 7, and on or after Day 7. In the framework of an imposed vaccination program, the ASCI administration may be postponed in order to administer the influenza vaccine on the fixed day. The ASCI administration should be given as close in time as possible to the time scheduled in the original administration schedule. However, a 6 day interval should be maintained between the influenza vaccine and the ASCI administration. The entire program of study visits and ASCI administrations will then be resumed (see Section 6.3.1).

5.6.4. Time window for recording concomitant medication and non-drug therapies in the eCRF

All concomitant medications, including changes in chronic medication with the exception of vitamins and/or dietary supplements, administered at ANY time during the period starting with the administration of each dose of ASCI and ending 30 days after each dose of ASCI are to be recorded in the eCRF.

Any product not foreseen in the study protocol administered in the period beginning 30 days preceding each dose of ASCI and ending 30 days after each dose of ASCI is to be recorded in the eCRF.

Any investigational medication or vaccine administered throughout the study (i.e. from first ASCI administration through to 30 days after the last ASCI) must be recorded in the eCRF.

Chronic medication will be recorded at the first study visit (Visit 1). Chronic medication is defined as medication taken by the patient for a minimum of 6 months before the study start.

5.7. Intercurrent medical conditions that may lead to elimination from the ATP cohort for analysis

Patients may be eliminated from the ATP cohort for analysis of immunogenicity if, during the study, they incur a condition that may alter their immune response.

6. STUDY ASSESSMENTS AND PROCEDURES

6.1. Patient identification and randomization of treatment

6.1.1. Patient identification

Patient numbers will be assigned sequentially to patients consenting to participate in the study, and according to the range of patient numbers allocated to each study center.

At the beginning of the study each investigation site will be given a range of patient numbers for that site. Patient numbers will be allocated consecutively at the site, from the range provided, in ascending order from the time of signing the informed consent. After signing the ICF, the investigator will enter the patient into the screening section of the eCRF. General demographic details and tumor characteristics will be entered in the screening section for each patient for whom a tumor sample is sent for analysis of NY-ESO-1 expression. When a patient is eligible (i.e., meets all the inclusion criteria and does not meet any of the exclusion criteria), the investigator or an authorized site staff member will continue with the actual eCRF for this patient.

6.1.2. Randomization / treatment assignment

This is a single treatment group study, so patients will not be randomized.

The treatment block lists will be prepared at GSK Biologicals, Rixensart, using MATEX, a program developed for use in *SAS*® (Cary, NC, USA) by GSK Biologicals.

The study product doses will be distributed to the study centers within pre-defined blocks.

The treatment allocation at the investigational site will be performed using a central allocation system on internet (SBIR; available 24hrs/day, 7 days/week).

Please refer to the SBIR user guide for specific instructions.

After having checked the eligibility of the patient, the site staff in charge of the ASCI administration will access the allocation system on internet. Upon providing the patient identification number and date of birth, the allocation system will determine the treatment numbers to be used for the patient. Each treatment number must be recorded in the eCRF on the Study Treatment Administration screen.

This procedure will be repeated for each subsequent dose allocation.

The treatment of the first five patients will occur in a staggered manner as described in Section 6.3.2.

6.2. Method of blinding

This study is an open-label single-group Phase I study, therefore, there will be no study blind.

6.3. General study aspects

6.3.1. Attendance of study visits

Patients will be required to attend for ASCI administrations and supplementary assessments according to the schedules shown in Section 6.4.

It is the investigator's responsibility to ensure that the stipulated intervals between study visits are strictly adhered to. Permitted deviation from the stipulated date of each visit (due e.g. to week-ends or public holidays) will be as follows:

Table 3 Permitted deviations from stipulated dates of visits

Cycle 1:	± 3 calendar days
Cycle 2:	± 3 calendar days
Cycle 3:	± 4 calendar days
Cycle 4:	± 7 calendar days

(Amended 03 September 2014)

Any deviation greater than this will be regarded as a protocol violation to be recorded in the eCRF.

In each case, the Sponsor will decide whether the violation is to be regarded as:

- A minor violation (i.e., without consequence),
- A major violation (resulting in exclusion of the patient from the population of patients evaluable according to protocol),
- A violation necessitating immediate exclusion of the patient from the study.

When the permitted deviations are applied or when a ASCI treatment has to be postponed (refer to Section 5.5.1 for details on postponement of study treatment), the entire program of study visits and ASCI administrations will be resumed as soon as the patient's condition allows or at the investigator's discretion. The visits following the first visit after a deviation or a postponement must be rescheduled on the basis of the actual date of this first visit, while respecting the stipulated time periods between study visits. Table 4 shows an example of how such a postponement causes a delay, which will be propagated to the schedule of visits and dose administrations throughout the entire study period.

Table 4 Example of postponement of study treatment dose for 3 weeks during Cycle 1 and the delay of the schedule induced

	Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Initial schedule	Visit	1		2		3		4		5		6		7			
	Dose	1		2		3		4		5		6					
Actual schedule due to postponement	Visit	1		2		X			3		4		5		6		7
	Dose	1		2		X			3		4		5		6		

Dose postponed for 3 weeks

All following visits and doses delayed for 3 weeks

Note: This postponement will have a similar impact on the complete schedule of visits and dose administrations throughout the entire study treatment period. Any further postponement(s) will induce additional delay(s) that must be added. ***(Amended 03 September 2014)***

If the re-treatment criteria are not met within 4 weeks after the scheduled treatment date (3 months during treatment Cycle 4), the patient must be withdrawn from the ASCI treatment and the procedures detailed for the Concluding Visit have to be carried out (see Section 6.5.4). Such patients will also be followed for survival for 5 years after the first ASCI administration (see Section 3.1). Table 5 illustrates this possibility.

Table 5 Example of postponement of ASCI treatment for more than 4 weeks leading to withdrawal of patient from the study treatment

	Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Initial schedule	Visit	1		2		3		4		5		6		7			
	Dose	1		2		3		4		5		6					
Actual schedule due to postponement	Visit	1		2		X					3						
	Dose	1		2		X					X						

Postponement of dose for more than 4 weeks → Patient withdrawn from study treatment and procedures of Concluding visit performed at Visit 3

Note: During Cycle 4 the maximum allowed postponement of a scheduled dose is 3 months and not the 4 weeks applicable to Cycles 1 – 3. If the re-treatment criteria are not met before the end of this period, the patient must be withdrawn from the study treatment.

6.3.2. Staggered enrolment of patients

The first five patients will be enrolled in a staggered manner controlled by a central process such that they will receive their first ASCI administration on different days. For example, one patient immunized per day until five patients have been immunized. The other patients can receive their first ASCI administration at any time starting from one day after the fifth patient has received his/her first ASCI administration.

6.3.3. Temporary or permanent stopping of the entire study

Further enrolment will be discussed between the Data Safety Monitoring Committee (DSMC; see Section 12.3) and GSK Biologicals' Safety Review Team (SRT; see Section 12.4) if:

- 4 patients develop severe toxicity (See Section 11.1.1 for the definition);

  or

- 2 or more Grade 4 SAEs or death occur that are definitely, probably or possibly related to NY-ESO-1 ASCI in 2 separate patients.

The respective National Competent Authorities and Institutional Review Boards (IRBs) will be notified of the outcome of this review.

6.4. Outline of study procedures

Study participation starts with a screening procedure. Signature of the ICF for testing of tumor tissue for NY-ESO-1 expression, gene profiling and for study participation will take place at the start of this screening procedure. The patient will also be proposed to sign a part of the Informed Consent Form allowing optional translational research on biological tissues sampled as part of the mandatory procedures of the trial.

The timing of scheduled assessments for the 4 ASCI administration cycles is shown in Table 6 to Table 9. ***(Amended 03 September 2014).***

All chest and complete-abdomen CT scans should be performed so that the results are available at the actual visit as per the tables below.

Table 6 List of study procedures for Cycle 1 (including screening)

Visit no.	Screening [a]	1	2	3	4	5	6	7
Intervals between study treatment administrations [h]		***2 weeks***						
Weeks after first ASCI administration	-	0	2	4	6	8	10	12
NY-ESO-1 ASCI admin no.	-	1	2	3	4	5	6	
Informed consent	●							
Addendum to informed consent [i]		●	●	●	●	●	●	●
Biopsy for NY-ESO-1 expression	●							
Inclusion/exclusion criteria	●	●						
Medical history	●							
Clinical evaluations								
Physical examination [b]	●	●	●	●	●	●	●	
Imaging procedures	● [c]							● [c]
Tumor response assessment								●
Safety assessments								
(Serious) Adverse Events recorded[d]	●	●	●	●	●	●	●	●
Potential immune-mediated diseases (pIMDs) recorded		●	●	●	●	●	●	●
Laboratory assessments								
Pregnancy test [e]	●	●	●		●		●	
Urine chemistry tests [f]	●						●	
HIV status [g]	●							
Hematological tests [f]	●	●		●			●	
Blood chemistry tests [f]	●	●		●			●	
Autoimmunity tests [f]	●						●	
Coagulation tests [f]	●	●		●			●	
Criteria for permanent stopping or postponement of study treatment			●	●	●	●	●	
Recording of concomitant medication		●	●	●	●	●	●	●

Note: For patients who are found to be NY-ESO-1 negative, only the following procedures need to be performed for this protocol: Informed consent, tumor biopsy, NY-ESO-1 expression testing, inclusion and exclusion criteria. All other procedures should only be performed if clinically indicated and do not need to be recorded on the eCRF.

a. Screening visit/period is to take place a maximum of 6 weeks before the first ASCI administration. However:
   1. Urine and blood chemistry, hematological, coagulation and autoimmunity tests, within 3 weeks before the first study treatment administration;
   2. Negative pregnancy test must be obtained within 1 week before the first study treatment administration for women of child-bearing potential;
   3. Tumor biopsy can be obtained within 12 weeks before the first ASCI administration; in the rare case the analysis of the sample would give an inconclusive result, the patient may be asked to allow a new sample to be collected or to allow the use of an already preserved sample to repeat the test. A tumor biopsy performed in the context of institution standard practice or another research study may be used for the NY-ESO-1 testing after obtaining the Sponsor's agreement, provided this biopsy was preserved in *RNAlater*® and was taken no more than 12 weeks before the first administration of the study treatment.

4. Tumor imaging no more than 6 weeks before first ASCI administration. Imaging performed as part of institution standard clinical practice or in relation to another research study does not need to be repeated, provided this imaging was performed no more than 6 weeks before the first administration of the study treatment.
5. HIV test within 3 weeks before the first ASCI administration, mandatory in Germany only (refer to Section 13.2).

b. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit***). Recording of all changes in the patient's medical condition including all adverse events.
c. Chest computer tomography (CT) scan, complete-abdomen CT scan and any other examination medically indicated to assess tumor dissemination. A contrast-enhanced CT scan or magnetic resonance imaging (MRI) of the brain is mandatory at the Screening Visit; while at subsequent visits it should only be performed in case of evocative neurological symptoms. Color photography or ultrasound of skin/node/subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will need to be sent to the site according to GSK PII management procedure.***
d. Refer to Section 9.3. At the screening visit only SAEs related to study participation and GSK concomitant medication will be recorded.
e. For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained at screening, before the first ASCI administration and after 2, 6 and 10 weeks of treatment (at Visits 1, 2, 4 and 6) to allow continued recNY-ESO-1 + AS15 administrations.
f. Refer to Section 6.7.2.
g. HIV test is mandatory to determine the HIV status (positive - negative) of patients in Germany only. Patients with known HIV-positive status do not need to be re-tested. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment (refer to Section 13.2).
h. In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administrations" rather than by the "weeks after first study treatment administration".
i. ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

(Amended 03 September 2014)

Table 7 List of study procedures for Cycle 2

Visit no.	8	9	10	11	12	13	14	15
Intervals between study treatment administrations[f]	***3 weeks***							
Weeks after first ASCI administration[f]	14	17	20	22	23	26	29	31
NY-ESO-1 ASCI admin no.	7	8	9		10	11	12	
Addendum to informed consent[g]	●	●	●	●	●	●	●	●
Clinical evaluations								
Physical examination [a]	●	●	●		●	●	●	
Imaging procedures [b]				●				●
Tumor response assessment				●				●
Safety assessments								
(Serious) Adverse Events recorded [c]	●	●	●	●	●	●	●	●
pIMDs recorded	●	●	●	●	●	●	●	●
Laboratory assessments								
Pregnancy test [d]	●	●	●		●	●	●	
Urine chemistry tests [e]	●		●				●	
Hematological tests [e]	●		●				●	
Blood chemistry tests [e]	●		●				●	
Autoimmunity tests [e]							●	
Coagulation tests [e]	●		●				●	
Criteria for permanent stopping or postponement of study treatment	●	●	●		●	●	●	
Recording of concomitant medication	●	●	●	●	●	●	●	●

a. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit).*** Recording of all changes in the patient's medical condition including all adverse events.

b. At Visits 11 and 15: chest, complete-abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will be sent to the site according to GSK PII management procedure.***

c. Refer to Section 9.3.

d. For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained before each ASCI treatment (at Visits 8-10 and 12-14) to allow continued recNY-ESO-1 + AS15 administrations.

e. Refer to Section 6.7.2.

f. ***In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administrations" rather than by the "weeks after first study treatment administration".***

g. ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

(Amended 03 September 2014)

Table 8 List of study procedures for Cycle 3

Visit no.	16	17	18	19	20
Intervals between study treatment administrations [g]	***6 weeks***				
Weeks after first ASCI administration [g]	33	39	45	51	54
NY-ESO-1 ASCI admin no.	13	14	15	16	
Addendum to informed consent [h]	●	●	●	●	●
Clinical evaluations					
Physical examination [a]	●	●	●	●	
Imaging procedures [b]					●
Tumor response assessment					●
Safety assessments					
(Serious) Adverse Events recorded [c]	●	●	●	●	●
pIMDs recorded	●	●	●	●	●
Laboratory assessments					
Pregnancy test [d]	●	●	●	●	
Urine chemistry tests [e]	●	●	●	●	
Hematological tests [e]	●	●	●	●	
Blood chemistry tests [e]	●	●	●	●	
Autoimmunity tests [e]				●	
Coagulation tests [e]	●	●	●	●	
Criteria for permanent stopping or postponement of study treatment	●	●	●	●	
Recording of concomitant medication	●	●	●	●	●
Conclusions for main analysis[f]					●

a. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit). Recording*** of all changes in the patient's medical condition including all adverse events.

b. At Visit 20: chest, complete-abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will be sent to the site according to GSK PII management procedure.***

c. Refer to Section 9.3.

d. For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained before each ASCI treatment (at Visits 16-19) to allow continued recNY-ESO-1 + AS15 administrations.

e. Refer to Section 6.7.2.

f. Main analysis will be performed when all patients have either completed the treatment up to Cycle 3 or have withdrawn from study treatment.

g. In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administrations" rather than by the "weeks after first study treatment administration".

h. ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

(Amended 03 September 2014)

Table 9 List of study procedures for Cycle 4

Visit no.	21	22	23	24	25	26	27	28	29
Date of Visit	V 19 + 3 m	V 21 + 3 m	V 22 + 3 m	V 23 + 3 m	V 24 + 6 m	V 25 + 6 m	V 26 + 6 m	V 27 + 6 m	V 28 + 30 days [a]
NY-ESO-1 ASCI admin no.	17	18	19	20	21	22	23	24	Concluding visit [b]
Addendum to informed consent [h]	●	●	●	●	●	●	●	●	
Clinical evaluations									
Physical examination [c]	●	●	●	●	●	●	●	●	●
Imaging procedures [d]		●		●	●	●	●		●
Tumor response assessment		●		●	●	●	●		●
Safety assessments									
(Serious) Adverse Events recorded [e]	●	●	●	●	●	●	●	●	●
pIMDs recorded	●	●	●	●	●	●	●	●	●
Laboratory assessments									
Pregnancy test [f]	●	●	●	●	●	●	●	●	
Urine chemistry tests [g]		●		●	●	●	●	●	●
Hematological tests [g]	●	●	●	●	●	●	●	●	●
Blood chemistry tests [g]	●	●		●	●	●	●	●	●
Autoimmunity tests [g]		●		●	●	●	●	●	●
Coagulation tests [g]		●		●	●	●	●	●	●
Criteria for permanent stopping or postponement of study treatment	●	●	●	●	●	●	●	●	
Recording of concomitant medication	●	●	●	●	●	●	●	●	●
Study conclusion									●

m = month, as calculation convention: 1 month is defined as 4 weeks i.e. 28 days during the treatment phase.

a. The procedures of the concluding visit are also to be carried out when a patient is withdrawn from study treatment.
b. For patients who have withdrawn because of disease progression, any resected tumor can be sent to GSK for analysis of the gene profile and other appropriate tests upon signature of the adequate ICF by the patient (refer to Section 12.2).
c. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit).*** Recording of all changes in the patient's medical condition including all adverse events.
d. At Visits 22, 24, 25, 26, 27 and 29: Chest, complete-abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory and must be performed within 2 weeks before the corresponding ASCI administration/visit. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions mustalso be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will be sent to the site according to GSK PII management procedure.***
e. Refer to Section 9.3.
f. For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained before each ASCI treatment (at Visits 21-28) to allow continued recNY-ESO-1 + AS15 administrations.
g. Refer to Section 6.7.2.

h. ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

(Amended 03 September 2014)

6.5. Detailed description of study procedures

6.5.1. Procedures prior to study participation

6.5.1.1. Informed consent

Before performing any other study procedure, the signed informed consent of the patient needs to be obtained. Refer to Section 12.2 for information on how to obtain informed consent.

The patient will also be proposed to sign the declaration of informed consent for translational and additional research (tick boxes on the informed consent form). This research is optional and the patient's decision to consent or not to allow translational research to be performed on his/her biological samples does not influence the eligibility of the patient for the study.

6.5.2. Procedures during screening phase/prior to the first ASCI administration

6.5.2.1. NY-ESO-1 expression screening of tumor lesion(s)

A tumor biopsy will be performed on the day of the patient's signature of the ICF or as soon as possible thereafter.

A tumor sample will be immediately stored in *RNAlater*® or equivalent RNA stabilizing solution (provided by GSK) and shipped as explained in the SPM to GSK Biologicals for the analysis of the expression of the NY-ESO-1 gene (see Section 6.7.3.1).

Note: In patients with sufficient tumor material (from the same lesion), another part of the tumor tissue will be formalin-fixed and paraffin-embedded (FFPE sample; a minimum of 4 mm^3 tumor sample must be preserved in *RNAlater*® and a minimum of 7 mm^3 is needed for the FFPE sample).

The tumor biopsy could have been done previously in the context of local routine practices or as part of another research study. This tumor material may be used for the NY-ESO-1 expression analysis if and only if:

- The patient signs the study ICF before the tumor sample is sent to GSK Biologicals for analysis, and;
- The first ASCI administration is to take place no more than 12 weeks after this tumor biopsy.

- The Sponsor's permission to use this tissue has been obtained in advance.
- The tumor tissue has been preserved in ***RNAlater®*** immediately after the surgery and the amount of tissue available must comply with the protocol requirements (see the SPM). ***(Amended 03 September 2014).***

In the rare case the analysis of the tumor sample gives an inconclusive result, the patient may be asked to allow a new sample to be collected or to allow the use of an already preserved sample to repeat the test.

The patient will also be proposed to sign the declaration of informed consent for translational and additional research on the initial biopsy (tick box on the ICF for study participation). Refusal to participate in this optional research will involve no penalty or loss of benefits to which the patient would otherwise be entitled.

6.5.2.2. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

If a patient is enrolled while not meeting all inclusion criteria or while meeting any of the exclusion criteria, this must be reported in the eCRF as a protocol deviation.

6.5.2.3. Collect demographic data

Record the patient's demographic data such as date of birth, gender and race in the eCRF.

6.5.2.4. Medical history

Perform a history-directed medical examination and record any pre-existing conditions and signs and/or symptoms present in a patient prior to the start of the study in the eCRF. Treatment of any abnormality observed during this examination has to be performed outside this study according to local medical practice or by referral to an appropriate health care provider.

6.5.2.5. Physical examination

Perform a physical examination of the patient, including assessment of the ECOG performance status, body temperature and resting systolic/diastolic blood pressure.

6.5.2.6. Tumor imaging and assessment

No more than 6 weeks before the administration of the first ASCI dose: Chest CT scan, complete-abdomen CT scan, and contrast-enhanced CT scan or MRI of the brain, as well as any other examination medically indicated to assess the dissemination of the tumor.

Note: Tumor imaging performed as part of standard clinical practice or in relation to another research study does not need to be repeated, provided this imaging was done no more than 6 weeks before the first administration of the study treatment.

In order to enroll a patient in the trial, this tumor imaging should confirm that the patient is not presenting visceral metastasis. Color photography or ultrasound of skin/node/subcutaneous lesions must also be performed ***if applicable. (Amended 03 September 2014).***

A complete assessment of all target lesions will be conducted. Superficial cutaneous lesions must be documented by photography in metric notation by use of a millimeter ruler or calipers. ***(Amended 03 September 2014)***

6.5.2.7. Urine and blood collection

No more than 3 weeks before the administration of the first ASCI dose:

- Collect a urine sample for urine chemistry tests including microalbuminuria. A validated quantitative test can be used according to local practice; for each individual patient the same test method must be used for all assessments**.**
- Collect a blood sample for hematological tests, blood chemistry tests, autoimmunity tests, blood urea nitrogen (BUN) and coagulation tests:

a. Hematological tests: complete blood counts should be performed to include hemoglobin, hematocrit, total and differential white blood cells and platelets.

b. Complete blood chemistry survey including LDH, CK, total protein and albumin, creatinine, serum bilirubin, direct bilirubin, ASAT, ALAT, alkaline phosphatase, and gamma-glutamyl transpeptidase.

c. Autoimmunity tests: anti-nuclear antibody (ANA).

d. Coagulation tests: prothrombin time (PT), activated partial thromboplastin time (APTT).

Further details of analysis are provided in Section 6.7.2.

6.5.2.8. Pregnancy test

Female patients of childbearing potential are to have a pregnancy test at screening and visits 1, 2, 4 and 6 during Cycle 1. Subsequently, a pregnancy test must be made prior to any ASCI administration. The ASCI may only be administered if the pregnancy test is negative. Pregnancy tests should be performed according to current practice at the center.

6.5.2.9. HIV status

HIV test is mandatory in Germany only. Please, refer to Section 13.2.

6.5.2.10. Recording SAEs

Any fatal SAEs and SAEs related to study participation or to concomitant use of GSK medication(s) are to be recorded (see Section 9.3.1).

6.5.3. Procedures during ASCI administration phase

Note that some of the procedures to be performed during the ASCI administration period (such as the physical examination, tumor imaging and assessment, taking urine samples, taking blood samples for hematological tests, blood chemistry tests, autoimmunity tests and coagulation tests, and pregnancy tests) are also performed during screening and are described in Section 6.5.2.

All chest CT scans, complete-abdomen CT scans, and any other examinations medically indicated to assess tumor dissemination should be performed so that the results are available at the actual visit.

Please refer to Table 6 to Table 9 for the timing of the study procedures in Cycles 1 to 4 (Section 6.4).

6.5.3.1. Check inclusion and exclusion criteria

At Visit 1 only, check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3. If the patient at this point in time no longer meets all of the inclusion criteria or meets any of the exclusion criteria, he/she must not be administered the study treatment and must be withdrawn from the study.

6.5.3.2. Check and record concomitant medication

Concomitant medication must be recorded in the eCRF as described in Section 5.6.2. Refer also to Section 5.6.2.1 for details on the medication forbidden and/or allowed during the study.

At each study visit subsequent to the first study product administration, it must be verified if the patient has experienced or is experiencing any concurrent medical condition(s) mentioned in Section 5.7. If this is the case, the condition(s) must be recorded in the eCRF.

6.5.3.3. Check contraindications to ASCI administration

Contraindications to ASCI administration and possible reasons for postponing the treatment are to be checked at the beginning of each study product administration visit. Refer to Section 5.5.

6.5.3.4. Blood sampling for safety or immune response assessments

As specified in the lists of study procedures in Section 6.4, blood samples are to be taken during certain study visits. Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

As of Protocol Amendment 3, no more blood samples will be collected for research purpose, i.e. blood samples taken for humoral immunological response and PBMC collection. For each blood sample already collected in the scope of this study and not tested yet, testing will not be performed by default, except if a scientific rationale remains relevant. Blood samples for safety assessment will continue to be collected and analysed. (Amended 03 September 2014).

6.5.3.5. Sampling of other body fluids

Urine sample: a volume of 5 mL is required for pH, protein, glucose, blood cells and densitometric examination of the urinary sediment for microalbuminuria.

6.5.3.6. Additional tumor biopsy or tumor resection

In case the investigator performs an additional tumor biopsy or tumor resection during the ASCI administration phase (see Section 5.6.1), sample(s) of the resected tumor lesion(s) will be sent to GSK Biologicals or a laboratory contracted for this by GSK Biologicals (see Sections 7.1 and 7.2.2). For patients who have given their specific informed consent to the optional translational research, such tumor tissue(s) may also be used to conduct these specific tests (see Sections 7.3, 7.6, 7.7, and 7.8).

Information will be provided regarding the additional resected/biopsied lesion(s). This will include the nature of the lesion (skin, lymph node, other), if the lesion is new or not, if the lesion is part of the target lesions or not, if the lesion is inflammatory or not, if vitiligo is present around the lesion or not and the status of the lesion when resected/biopsied (progressive, stable or regressive).

6.5.3.7. Treatment number assignment

At each ASCI administration visit, the patient will be assigned a treatment number defining the treatment he/she will be receiving. The treatment number must be recorded in the eCRF at each ASCI administration visit.

6.5.3.8. ASCI administration

The patient will receive the ASCI (i.m in the deltoid or the lateral region of the thigh; see Section 5.3.2). If the investigator or delegate decides that the patient's health on the day of ASCI administration temporarily precludes ASCI administration, the visit will be rescheduled within the postponement interval for this visit (Section 6.3.1).

The patient will be observed closely for at least 30 minutes, with appropriate medical treatment readily available in case of anaphylaxis following the administration of the ASCI.

6.5.3.9. Recording of non-serious AEs, SAEs, potential immune-mediated diseases (pIMDs) and pregnancy

Refer to Section 9.3 for procedures for the investigator to record AEs and SAEs and to Section 9.4 for guidelines on how to report these AEs/SAEs to GSK Biologicals. Refer to Section 9.4.5 for instructions on reporting of pIMDs.

The patients will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.5.3.10. Tumor response assessment

Based on the tumor imaging and (adapted) RECIST criteria (as described in Section 6.6), the tumor response will be assessed and recorded.

6.5.3.11. Clinical response criteria for continuing ASCI administration

Unlike chemotherapy that may cause prompt clinical tumor shrinkage already after 2 to 4 weeks by exerting a direct cytotoxic effect on tumor cells, ASCIs function indirectly by eliciting a cellular and humoral immune response, which takes time to develop and is maximal only several weeks after the initial immunizations. In accordance with this, in previous and ongoing trials with cancer immunotherapeutics most clinical responses were observed several weeks after the beginning of the immunizations. Moreover, long-term maintenance treatment with immunizations was followed in some patients, who initially showed stable disease or mixed response, by long-term complete tumor response. Therefore, in the present study, the patient's response to treatment will be assessed during and after each cycle to determine whether to continue treatment.

The overall tumor responses to treatment qualifying patients to receive up to a maximum of 24 ASCI administrations are:

- Objective responses: Complete Response (CR) and Partial Response (PR).
- Stable Disease (SD).
- Slow Progressive Disease (SPD).

SPD (defined in Section 6.6.6) corresponds to a protocol-specific convention allowing the continuation of ASCI administrations even in the event of a Progressive Disease (PD).

Only patients with CR, PR, SD and SPD at the first tumor response evaluation at the end of Cycle 1 (Visit 7) may continue to receive study treatment. All the other patients will not be admitted to treatment in Cycle 2. Refer to Section 6.6 for the definitions of tumor response.

The patients will be assessed midway through Cycle 2 (Visit 11). Patients showing CR, PR, SD and SPD may continue to receive study treatment, while all the other patients must discontinue the treatment.

Patients showing CR, PR, SD and SPD at the third tumor response evaluation at the end of Cycle 2 (Visit 15) may continue to receive study treatment in Cycle 3. All the other patients will not be admitted to treatment in Cycle 3.

Patients showing CR, PR, SD and SPD at the end of Cycle 3 (Visit 20), will be admitted to treatment in Cycle 4. All the other patients must discontinue treatment.

Patients showing CR, PR, SD and SPD at Visits 22, 24, 25, 26 and 27 (Section 6.6) will be allowed to continue Cycle 4.

6.5.4. Concluding visit

6.5.4.1. Patients who received all the scheduled doses

The concluding visit will take place 30 days after Visit 28 (for patients withdrawn from the study treatment before completion, see Section 6.5.4.2).

The following procedures will be performed within 2 weeks of this visit:

Chest CT scan, complete-abdomen CT scan, and any other examination medically indicated to assess tumor dissemination. A contrast-enhanced CT scan or MRI of the brain should only be performed in case of evocative neurological symptoms. Color photography or ultrasound of skin/node/subcutaneous lesions must also be performed ***if applicable. (Amended 03 September 2014).***

Based on the tumor imaging and RECIST criteria (as described in Section 6.6), the overall tumor response will be assessed.

At the visit, the following procedures described above will be performed: physical examination; recording any AEs and SAEs, pIMDs, and concomitant medications; taking a urine sample for urine chemistry tests; taking a blood sample for hematological tests, blood chemistry tests, autoimmunity tests, coagulation tests. ***(Amended 03 September 2014).***

6.5.4.2. Patients withdrawn before the end of the scheduled doses

The patients are free to withdraw from the study at any time and for any reason without this having any prejudicial impact on their further treatment. All patients who have received any dose of the study treatment should be followed for toxicity assessment.

For patients whose participation in the study is discontinued - for whatever reason - before all the scheduled ASCI treatments have been administered, the concluding visit is to be carried out at least 30 days after the patient's last dose of ASCI.

The following procedures will be performed no more than two weeks before this visit. If imaging has been performed less than ***6*** weeks before, this need not be repeated ***(Amended 03 September 2014)***:

- Chest CT scan, complete-abdomen CT scan, and any other examination medically indicated to assess tumor dissemination. A contrast-enhanced CT scan or MRI of the brain should only be performed in case of evocative neurological symptoms.

During the visit, the following procedures described above will be performed: physical examination including the recording of the ECOG performance status; recording any (serious) AEs, pIMDs and concomitant medications; taking a urine sample for urine chemistry tests; taking a blood sample for hematological tests, blood chemistry tests, autoimmunity tests, coagulation tests. ***(Amended 03 September 2014).***

The urine and blood sampling associated with the concluding visit must be performed before initiation of any other new systemic anticancer treatment. If necessary, these samplings will therefore be performed separately from the other procedures of this concluding visit.

For patients who have withdrawn because of progression of the disease, any resected tumor can be sent to GSK for analysis of NY-ESO-1 expression and gene profile upon signature of the adequate ICF by the patient (refer to Section 12.2); for patients who have given their specific consent to this, such tumor tissue(s) may also be used for optional translational research.

6.5.4.3. Study conclusion

The study conclusion will be made at ***the Concluding Visit***. ***(Amended 03 September 2014).***

6.6. Response criteria

Some patients may present an objective tumor response that may be evaluated according to the RECIST criteria [Therasse, 2000] as the primary endpoint of this study (see Section 6.6.1).

Nevertheless, the majority of the patients of the study population will present a cutaneous and/or sub-cutaneous and/or lymph node disease which may not be assessed according to the RECIST criteria, because all target lesions will have a longest diameter < 20 mm (in-transit metastasis, Stage IV M1a disease). Specific measurability and response criteria must therefore be defined for this particular subgroup of patients (see Section 6.6.2).

A third category of patients may present both target lesion(s) ≥ 20 mm and target lesion(s) < 20 mm. Specific measurability and response criteria must also be defined for this subgroup of patients (see Section 6.6.3).

6.6.1. Response criteria for patients with all target lesions ≥ 20.0 mm

Objective tumor response is assessed according to the RECIST criteria as a primary endpoint of this study.

The response assessment is essentially based on a set of measurable lesions identified at baseline as target lesions, and followed until disease progression.

The following paragraphs contain a brief summary of the RECIST criteria [Therasse, 2000] as appropriate to this study. The complete criteria are available at http://www.eortc.be/recist/documents/RECISTGuidelines.pdf.

6.6.1.1. Measurability of tumor lesions

6.6.1.1.1. Definitions

Measurable disease: the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions: lesions that can be accurately measured in at least one dimension with longest diameter ≥ 20 mm. With spiral CT-scan, the lesion must be 10 mm in at least one dimension.

Non-measurable lesions: all other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and other non-measurable lesions. These include: bone lesions; leptomeningeal disease; ascites; pleural / pericardial effusion; inflammatory breast disease; lymphangitis cutis / pulmonis; abdominal masses that are not confirmed and followed by imaging techniques; and cystic lesions.

All measurements should be recorded in metric notation by use of a ruler or calipers. All baseline evaluations should be performed as closely as possible to the start of treatment and never more than 6 weeks before the start of the treatment.

6.6.1.1.2. Methods of measurement

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline. ***(Amended 03 September 2014).***

- Clinically detected lesions will only be considered measurable when they are superficial (e.g. skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography - including a ruler to estimate the size of the lesion - is required.

- CT-scan and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT-scan and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT-scan should be performed using a 5 mm contiguous reconstruction algorithm; this specification applies to the tumors of the chest, abdomen and pelvis while head and neck tumors and those of the extremities usually require specific protocols.
- Ultrasound (US) should not be used to measure tumor lesions that are clinically not easily accessible. It may be used as a possible alternative to clinical measurements of superficial palpable nodes, subcutaneous lesions. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination. US should preferentially be performed by the same investigator.

6.6.1.2. Tumor response evaluation

6.6.1.2.1. Baseline documentation of target and non-target lesions

All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded and measured at baseline. Measurements are not required but the presence or absence of each should be noted throughout follow-up.

6.6.1.2.2. Response criteria

Evaluation of target lesions	
Complete Response (CR)	Disappearance of all target lesions.
Partial Response (PR)	At least a 30% decrease in the sum of LD of target lesions taking as reference the baseline sum LD.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.
Progressive Disease (PD)	At least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded since the treatment started OR the appearance of one or more new lesions OR both of these.

Evaluation of non-target lesions	
Complete Response (CR)	Disappearance of all non-target lesions.
Incomplete Response / Stable Disease	Persistence of one or more non-target lesion(s).
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. (Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail.)

6.6.1.2.3. Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

Summary for evaluation of overall response

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR ***(or NA)***	No	CR
CR	Incomplete response / SD	No	PR
PR	Any response other than PD	No	PR
SD	Any response other than PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Not all evaluated	***Any response other than PD***	***No***	***NE***
Any response other than PD	***Not all evaluated***	***No***	***NE***
Any	Any	Yes	PD

Abbreviations: CR: complete response; PR: partial response; SD: stable disease; PD : progressive disease; NA: not applicable; NE: non-evaluable.

(Amended 03 September 2014).

6.6.2. Tumor response for cutaneous/subcutaneous/lymph node disease with target lesions of less than 20.0 mm

Some patients of this protocol will present a cutaneous and/or sub-cutaneous and/or lymph node disease that could not be evaluable according to RECIST criteria because all target lesions will have a longest diameter < 20 mm (in transit metastasis, Stage IV M1a). Specific measurability and response criteria must therefore be defined for this particular subset of patient.

Similarly to the standard RECIST criteria, the response assessment for patients with cutaneous and/or subcutaneous and/or lymph node disease with target lesions of less than 20 mm are essentially based on a set of measurable lesions identified as target lesions at baseline, and followed until disease progression.

6.6.2.1. Measurability of cutaneous/subcutaneous/lymph node tumor lesions at baseline

6.6.2.1.1. Definitions

Measurable disease: the presence of at least one cutaneous or subcutaneous or lymph node measurable lesion.

Measurable cutaneous/subcutaneous/lymph node lesions: lesions that can be accurately measured in at least one dimension.
Minimal longest diameter required for one target lesion is:

	Clinical detection (color photography)	**Ultrasound**
Cutaneous lesion	Lesion must be ≥ 5 mm	–
Subcutaneous lesion	–	Lesion must be ≥ 5 mm
Lymph-node lesion	–	Lesion must be ≥ 5 mm

Measurable cutaneous confluent lesion: if cutaneous lesions are too numerous and too small (< 5 mm) but are confluent, they can be recorded as a specific measurable confluent lesion.

Non-measurable lesion: all other lesions, including small lesions (longest diameter < 5 mm).

All measurements should be recorded in metric notation by use of a ruler or calipers. Baseline evaluations should be performed as closely as possible to the start of treatment (could be performed at Visit 1) and never more than ***6*** weeks before the start of the treatment. ***(Amended 03 September 2014).***

6.6.2.1.2. Methods of measurement

The same method of assessment and the same technique should be used to characterize each identified and reported (confluent) lesion at baseline and during follow-up.

For patients presenting a cutaneous disease with all lesions having a longest diameter of < 20 mm (measurable cutaneous lesions, measurable cutaneous confluent lesions and non-measurable lesions), the lesions must be documented/recorded by color photography - including a ruler to estimate the size of the lesion.

For patients presenting a subcutaneous or lymph node disease with all lesions having a longest diameter of < 20 mm, lesions must be documented/recorded by ultrasound (US) or CT scan. Note: US may also be useful to confirm the complete disappearance of cutaneous lesions usually assessed by clinical examination.

6.6.2.2. Tumor response evaluation

6.6.2.2.1. Baseline documentation of target and non-target lesions

All measurable cutaneous/subcutaneous/lymph node lesions and measurable cutaneous confluent lesion up to a maximum of 5 lesions per site (skin, subcutaneous or lymph node) should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate repetitive measurements (either by US or clinically).

All other lesions should be identified as non-target lesions and should also be recorded and evaluated at baseline ***as well as at each further visit including a tumor response assessment.*** They should be followed as "present" or "absent" ***at baseline and followed using the RECIST criteria at further visits. Independently to this follow-up assessment, any new lesion will be recorded as non-target lesion. (Amended 03 September 2014).***

6.6.2.2.2. Response criteria

Evaluation of target lesions

Complete Response (CR)	Disappearance of all target lesions.
Partial Response (PR) / Stable Disease (SD)	Neither sufficient shrinkage to qualify for CR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.
Progressive Disease (PD)	According to investigator, clear increase of diameters of target lesions taking as references the smallest diameters recorded since the treatment started OR the appearance of one or more new target lesions OR both of these.

Evaluation of non-target lesions

Complete Response (CR)	Disappearance of all non-target lesion.
Incomplete Response / Stable Disease	Persistence of one or more non-target lesion(s).
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. (Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail.)

6.6.2.2.3. Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

6.6.3. Response criteria for patients with both target lesion(s) ≥ 20 mm and target lesion(s) < 20 mm

The present guidelines prevent the consideration of a patient with good responding lesions ≥ 20 mm as progressive based on progression of some small lesions (< 20 mm) or to consider a patient with one large stable lesion (≥ 20 mm) as stable disease while all the other target lesions (< 20 mm) show progression.

The advice for response evaluation in this patient category is to take the sum of the longest diameters for all target lesions (< and ≥ 20 mm) and to use the response criteria as defined below.

The definitions for complete response (CR), partial response (PR) and stable disease (SD) remain the same.

Progressive disease can be defined as either an increase of at least 20% in the sum of the longest diameters of the target lesions OR if at least half of the target lesions show at least a doubling of diameters OR the appearance of one or more new lesions.

Evaluation of target lesions	
Complete Response (CR)	Disappearance of all target lesions.
Partial Response (PR)	At least a 30% decrease in the sum of LD of target lesions taking as reference the baseline sum LD.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.
Progressive Disease (PD)	At least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded since the treatment started OR the appearance of one or more new lesions OR both of these.

Evaluation of non-target lesions	
Complete Response (CR)	Disappearance of all non-target lesions.
Incomplete Response / Stable Disease	Persistence of one or more non-target lesion(s).
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. (Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail.)

6.6.3.1. Evaluation of best overall response

See corresponding part of Section 6.6.1.2.2.

6.6.4. Confirmation measurements and duration of response

6.6.4.1. Confirmation

The main goal of confirmation of objective response is to minimize the risk of overestimation of the response rate. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed. In the present study, responses always need to be confirmed.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeated assessments that should be performed no less than 4 weeks after the criteria for response are first met.

6.6.4.2. Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is recorded first) until the first date that recurrence or PD is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started).

6.6.4.3. Duration of stable disease

Stable disease (SD) is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the sum of LD of target lesions recorded previously but not necessarily at baseline.

The minimal time interval required between two measurements for determination of SD is at least 16 weeks.

6.6.5. Definition of mixed response

The concept of a Mixed Response (MR) is based on the observation that some melanoma patients present with tumor lesions that indubitably regress after ASCI administration, whereas other lesions remain unchanged or progress or with new lesions appearing.

As one example, consider a case with the complete regression of all initial target lesions but with the appearance of one new lesion - this must certainly be considered as a PD. However, it is important that the detailed information about such a case is recorded in an early clinical study as a MR, because such a situation could be explained by an adequate biological activity of the ASCI but with a resistance of the new tumor lesion to the immune response induced by the ASCI (i.e., HLA Class I loss, NY-ESO-1 negative metastasis).

Cases of MR will NOT be considered as meeting the criteria of the definition of an objective response (for assessment of the primary endpoint), as all MR are either a stable disease (SD) or a progressive disease (PD). However, the recorded information about MRs will be useful in the descriptive assessment of the biological activity of the ASCI treatment.

6.6.5.1. Mixed response adapted from the RECIST criteria

Some patients will present an objective tumor response that may be measured according to the RECIST criteria. For these patients, the MR criteria are defined as follows:

- At least 30% decrease in the longest diameter (LD) occurring in at least one target lesion recorded and measured at baseline. Such response occurring in otherwise SD or PD status of the LD of target lesions and without the appearance of one or more new lesions will be classified as “SD with target lesion regression” or “PD with target lesion regression”, respectively.
- The appearance of new lesions in otherwise PR status of the LD of target lesions will be classified as “PR with new lesion.

6.6.5.2. Mixed response for disease not evaluable according to RECIST criteria

Some patients of the target population of this study will present cutaneous and/or sub-cutaneous and/or lymph node lesion(s) that cannot be evaluated according to the RECIST criteria because all target lesions will have a longest diameter < 20 mm as in transit metastasis, (i.e., patients with Stage IV M1a disease).

For these patients, the MR criteria are defined as follows:

- A clear decrease of diameters occurring in at least one target lesion recorded and measured at baseline. Such response occurring in otherwise SD or PD status of the LD of (baseline) target lesions and without the appearance of one or more new lesions will be classified as “SD with target lesion regression” or “PD with target lesion regression”, respectively.

- The appearance of new lesions in otherwise PR status of the LD of target lesions will be classified as "PR with new lesion.***(Amended 03 September 2014).***

6.6.6. Definition of Slow Progressive Disease status

Unlike chemotherapy, which may prompt tumor shrinkage within 2 - 4 weeks of treatment start by exerting a direct cytotoxic effect on the tumor cells, ASCI therapies function indirectly by eliciting an immune response (humoral and cellular), which requires time and repeated immunizations to be established and hence becomes maximal only several weeks after the initiation of the ASCI injections. Thus, in previous and ongoing cancer immunotherapeutic trials, most clinical responses were recorded several weeks after the start of the immunizations. Moreover, in some patients with otherwise stable disease or a mixed response, long-term maintenance treatment with ASCI injections has been followed by a complete tumor response.

The concept Slow Progressive Disease (SPD) has been defined to allow for the time required for eliciting a stable and effective immune response and to avoid premature withdrawal from the study treatment of some patients who might benefit from continued immunization. For patients meeting the criteria for SPD status, disease progression is therefore not to be considered as a criterion for withdrawal from the study treatment.

It is to be noted that SPD is **not** to be considered as a success criterion of the study, and time to progression will be determined as the time of first evidence of disease progression notwithstanding that the patient may have been allowed to continue the study treatment because of having SPD status.

The SPD status is defined below:

Slow Progressive Disease	All of the following criteria must be met: 1. The patient's ECOG performance status is 0 or 1. 2. The patient's LDH value is not greater than twice the normal upper limit. 3. There is no appearance of visceral metastases other than in the lung. 4. The patient does not meet any of the criteria for permanent stopping of study treatment (Section 5.5.2).

6.7. Biological sample handling and analysis

As of Protocol Amendment 3, no more biological samples will be collected for research purpose. For each biological sample already collected in the scope of this study and not tested yet, testing will not be performed by default, except if a scientific rationale remains relevant. In this case, testing will be done in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below. (Amended 03 September 2014).

Please refer to the SPM for details of biospecimen management (handling, storage and shipment).

Samples will not be labeled with information that directly identifies the patients but will be coded with the identification number for the patient (patient number).

Collected samples may also be used for purposes related to the quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these current tests, the maintenance or improvement of these current tests, the development of new test methods for the markers described in this protocol, as well as making sure that new tests are comparable to previous methods and work reliably.

It may be that any findings in the present study or in other studies necessitate further investigation by GSK Biologicals into the efficacy or immunogenicity of the recNY-ESO-1 + AS15 ASCI and its constituents or further research in the disease under study. Therefore, all patients in countries where this is allowed, will be invited to give their separate agreement (tick box in the Informed Consent Form) to allow GSK to perform, under these circumstances, additional testing outside the scope of this protocol on the samples.

It is also possible that future findings may make it desirable to use the samples acquired in this study for tests that are not related to the disease under study. Therefore, all patients in countries where this is allowed, will be invited to give their separate agreement (tick box in the Informed Consent Form) to allow GSK to perform such additional testing.

The decision to accept or reject translational and/or additional testing will not affect the ability of the patient to enter the study. Any sample testing will be done strictly in line with the consent of the individual patient and will be subject to the laws and regulations concerning such testing in the respective countries. This additional research will only be performed once the relevant Ethics Committee(s) has/have been informed and has/have reviewed the tests to be performed, and has/have had the possibility of deciding to obtain the specific informed consent of the patient for this/these test(s).

Any sample testing will be done in line with the consent of the individual patient.

Any human pharmacogenetic testing will require specific consent from the individual patients and the ethics committee approval.

Refer also to the Investigator Agreement, where it is noted that the Investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for up to 15 years (counting from when the last patient performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the patient consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

6.7.1. Use of specified study materials

When materials are provided by GSK Biologicals or central laboratories contracted by GSK Biologicals, it is MANDATORY that all clinical samples be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the patient from the ATP analysis (See Section 11.4 for the definition of study populations to be evaluated). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, then appropriate materials from the investigator's site are to be used. Refer to the Module on Clinical Trial Supplies in the SPM or biospecimen management for more details.

6.7.2. Hematology, Serum Chemistry and Urine tests

Sampling for the various laboratory tests will be performed as shown in Table 10. For details of the blood sampling (volumes in each tube), information will be supplied to the study sites as necessary. These assays will be conducted at each investigational site's local laboratory; samples will be handled according to the site's standard procedures. If a clinically significant laboratory abnormality is detected at one of these assessments, it should be followed up as adequate until it has returned to normal or a satisfactory explanation has been provided.

Specification for Germany: An additional blood sample might be required for HIV test. Collect of the sample will be performed according to standard local practice. For more information, please refer to Section 13.2.

Table 10 Hematology, Serum Chemistry, Urine tests

Sample type	System	Component	Scale	Timing
HEMATOLOGY				
Blood (5 mL)	Whole blood	Hemoglobin Hematocrit Complete blood cell count Total and differential white blood cell count Platelets	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 21 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
BIOCHEMICAL VALUES				
Blood (5 mL)	Serum	Sodium Potassium Calcium Lactate dehydrogenase (LDH) Creatine kinase (CK) Total protein[4] Albumin[4]	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 21, 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
HEPATIC FUNCTION				
Blood (5 mL)	Serum	Aspartate aminotransferase (ASAT) Alanine aminotransferase (ALAT) Alkaline phosphatase Gamma-glutamyl transpeptidase (γGT) Serum bilirubin[4] Direct bilirubin[3,4]	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 21, 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
RENAL FUNCTION				
Blood (5 mL)	Serum	Blood urea nitrogen (BUN)[5] Creatinine	Quantitative	During screening Cycle 1: Visit 6 Cycle 2: Visits 10 and 14 Cycle 3: Visits 17 and 19 Cycle 4: Visits 22, 25, and 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
COAGULATION				
Blood (5 mL)	Serum	Prothrombin Time (PT) Activated Partial Thromboplastin Time (APTT)	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s)

Sample type	System	Component	Scale	Timing
AUTOIMMUNITY				
Blood (5 mL)	Serum	Anti-nuclear antibody (ANA) [1]	Quantitative	During screening Cycle 1: Visit 6 Cycle 2: Visit 14 Cycle 3: Visit 19 Cycle 4: Visits 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s) Follow-up Visits: F2, F4
PREGNANCY TESTING (MANDATORY) [2]				
Blood (5 ml) or urine according to local practice	Serum or urine	According to local practice	Ordinal	During screening Cycle 1: Visits 1, 2, 4, and 6 Cycles 2 - 4: prior to each ASCI administration
URINALYSIS				
Urine	Urine	Protein (test strip) Glucose (test strip)	Ordinal	During screening Cycle 1: Visit 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s)
		pH (test strip) Erythrocyte (Red Blood Cells - test strip) Microalbuminuria[3,4] (densitometric evaluation of urinary sediment)	Quantitative	

1. ANA test will be carried out by immunofluorescence on HEPG2 cells. In case anti-nuclear antibodies are detected, further analysis will be performed by immunodot and/or enzyme-linked immunosorbent Assay (ELISA) to identify the auto-target antigen.
2. For women of child-bearing potential only.
3. ***It is mandatory to measure direct bilirubin/microalbuminurea, only when bilirubin/albumin testing reveals abnormal results.***
4. ***A quantitative or qualitative test can be performed at the investigator's discretion. In cases where positivea qualitative results are received, test a quantitative test also needs to be performed. It is sufficient to do only one test for protein in Urine, if this test is sensitive enough.***
5. ***Measurement of serum urea (azotemia) is allowed instead of measurement of BUN. The choice between these tests is left at the investigator's discretion. If measurements are in serum urea, they need to be converted to BUN. The conversion has to made according to the following formula: Urea [mg/dL]= BUN [mg/dL] * 2.14. BUN can be expressed in either in mg/dL or in mmol/L. If Serum Urea has been expressed in mmol/L, the conversion factor is 1 and as the formula is: SU (mmol/L) = BUN (mmol/L).***

(Amended 03 September 2014)

6.7.3. Molecular, immunological and translational research read-outs

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected. (Amended 03 September 2014).

Table 11 summarizes the molecular and immunological read-outs that have to be performed during the study as well as translational research and provides the time points for performance of these tests. For a more detailed description of the read-outs and research refer to Sections 6.7.3.1, 6.7.3.2 and 7. The tests will be performed by GSK SK Biologicals or a validated laboratory designated by GSK Biologicals.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (Sponsor-dependent) but laboratory-independent Quality Department.

Table 11 Overview of molecular and immunological read-outs and translational research

***NY-ESO-1* SCREENING (MANDATORY)**			
Test	**Sample Type**	**Laboratory**	**Timing**
***NY-ESO-1* expression testing** (see also Section 6.7.3.1)	Fresh tissue sample (in *RNAlater*® or equivalent)	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.
IMMUNOLOGICAL READ-OUTS (MANDATORY)			
Test	**Sample Type**	**Laboratory**	**Timing**
Humoral immunity (see Section 6.7.3.2.1) Anti-*NY-ESO-1* antibody response	Serum (2 x 5 ml) [b]	GSK Biologicals or contracted lab [a]	Cycle 1: Visits 1, 3, 5 to 7 Cycle 2: Visit 14 Cycle 3: Visit 19 Cycle 4: Visits 22, 24 to 27 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s) Follow-up Visits: F1 to F4
Cellular immunity (see Section 6.7.3.2.2) Cellular (T-cell) response (including regulatory T-cell responses)	Blood (150 ml) [c]		Cycle 1: Visits 1 and 6 Cycle 2: Visit 14 Cycle 3: Visit 19 Cycle 4: Visits 22, 24 to 27 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s) Follow-up Visits: F2, F4
MANDATORY TRANSLATIONAL RESEARCH (SEE SECTION 7.2.1)			
Test	**Sample Type**	**Laboratory**	**Timing**
Gene profiling of the tumor	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d]	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.

OPTIONAL TRANSLATIONAL RESEARCH (SEE SECTION 7 EXCEPT 7.2.1)			
Test	**Sample Type**	**Laboratory**	**Timing**
Expression analysis of NY-ESO-1 and other tumor antigens (e.g., MAGE-A3, PRAME, LAGE-1, MAGE-C2 and WT1)	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d]	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.
***Natural immunogenicity and* Antigen spreading**	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.
			May be performed on any of the blood samples taken for the humoral and cellular immunity tests
Gene profiling of the immune response	Blood (150 ml) [c]		May be performed on any of the blood samples taken for the cellular immunity tests (PBMC collection)
Proteomic profiling	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy) or on any of the blood samples taken for the humoral and cellular immunity tests
infiltrating lymphocytes (including T regulatory cells and other immune cellular entities)	Either fresh tissue sample (in *RNAlater*® or equivalent) or FFPE tissue (if available) [d]		During screening (initial biopsy). In case of additional tumor biopsy or tumor resection
DNA methylation status of NY-ESO-1, other antigens and predictive genes, in addition to immunoregulatory genes	Either fresh tissue sample (in *RNAlater*® or equivalent) or FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy) or in case of additional tumor biopsy or tumor resection (disease progression), or on any of the blood samples taken for the humoral and cellular immunity tests
Pharmacogenetics analyses	Tumor samples and blood		Preferably on blood collected at Visit 1. On tumor cells from screening or from additional lesions

a. GSK Biologicals laboratory or a validated laboratory designated by GSK Biologicals.
b. 2 x 5 ml of blood will be used for both anti-*NY-ESO-1* antibody responses; and for the optional translational research (as applicable).
c. A total of 150 ml of blood will be used for cellular immunological read-outs; and for the optional translational research (as applicable).
d. FFPE tissue is only to be collected from patients with sufficient tumor material from the same lesion to provide a fresh sample and a FFPE sample. GSK Biologicals or a contracted laboratory will require a block of at least 7 mm^3 or 15 slides of 10 μm each.

(Amended 03 September 2014)

6.7.3.1. NY-ESO-1 expression screening of the tumor

This test will be performed on fresh tumor tissue. The tumor sample will be processed as described in the SPM.

Note that in the rare case that the analysis of the sample would give an inconclusive result, the patient may be asked to allow a new sample to be collected to repeat the test or, if available, to allow the use of an already preserved sample to repeat the test.

This test will be performed at GSK Biologicals or at another laboratory or laboratories chosen and contracted for this task by GSK Biologicals.

NY-ESO-1 expression screening of the tumor will be carried out using a real-time quantitative PCR test developed and validated by GSK or any updated technique at the time of analysis.

NY-ESO-1 transcript will be amplified in parallel with one or more control genes (e.g. β-actin), used for normalization and relative quantification of NY-ESO-1 transcript levels. Commercial RNA or RNA from cell lines expressing NY-ESO-1 is used as a positive control and commercial RNA or RNA from cell lines that do not express NY-ESO-1 is used as negative control.

A sample will be defined as NY-ESO-1 positive if NY-ESO-1 transcript level in extracted RNA (normalized to one or more control genes) is above a fixed cut-off level.

6.7.3.2. Immunological read-outs

As the immunotherapy proposed for this study is designed to activate both B- and T-cell compartments of the immune system, both the serology and the cellular immune responses induced by the immunization will be measured.

As this study is the first time in human study with the recNY-ESO-1 + AS15 ASCI, no serum or PBMC samples of immunized patients have been available so far to optimize the assays and to determine cut-off values to define the immunological responders. The optimal assays and cut-off values will be established during the course of this clinical study.

These tests will be performed at GSK Biologicals or at another laboratory or laboratories chosen and contracted for this task by GSK Biologicals.

6.7.3.2.1. Antibody response to the ASCI antigens

Currently, testing of serum to assess antibody mediated immune responses has been performed for a subset of samples collected in this study. Further testing will only be performed if a scientific rationale remains relevant in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below. (Amended 03 September 2014).

The antibody response to the NY-ESO-1 antigen will be assessed for all patients enrolled into the study. ***(Amended 03 September 2014)***

Specific anti-NY-ESO-1 antibodies ***pre-existing and*** induced by the recNY-ESO-1 + AS15 ASCI will be measured by ELISA or upgraded techniques, and the results will be provided in EU/mL or other appropriate units. This can be done using either the NY-ESO-1 recombinant protein or NY-ESO-1-derived peptides spanning the entire NY-ESO-1 sequence as coating antigen. A patient will be considered as seropositive if the antibody titer is superior or equal to the assay cut-off value and at least twice the patient's value at baseline. ***(Amended 03 September 2014).***

These tests will be performed at GSK Biologicals or at another laboratory chosen and contracted for this task by GSK Biologicals.

At each planned visit (as defined in Section 6.4), the amount of blood to be drawn for the analysis of antibody response will be 2 x 5 mL blood in appropriate tubes. Time points for serum collections are described in Section 6.4.

6.7.3.2.2. Cellular immune response to the ASCI antigens

Currently, testing of PBMCs to assess cell-mediated immune responses has been performed for a subset of samples collected in this study. Further testing will only be performed if a scientific rationale remains relevant in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below. (Amended 03 September 2014).

1. **PBMC processing and analysis**

The cell-mediated immune (CMI) response ***pre-existing or*** induced by the ASCI administrations will be assessed for all patients enrolled into the study***(Amended 03 September 2014)***.

The technique used for assessing CMI responses may be updated to the best available approved procedure at the time of analysis. These analyses will be completed (or performed again with an updated technique) at GSK Biologicals or at another laboratory chosen and contracted for this task by GSK Biologicals.

In order to obtain a more precise picture of the patient's CMI response, a Human Leukocyte Antigen (HLA) typing could be needed, but this would not require additional blood sampling.

Peripheral blood mononuclear cells (PBMC), to be drawn for the CMI response analysis at certain planned visits, will be obtained from 150 mL of venous blood. Time points for collection of blood for PBMC isolation are indicated in Section 6.4.

To ensure that the PBMC samples taken are usable, these visits must take place according to the schedule provided in the SPM. For details, please refer to the SPM.

PBMC processing will be performed centrally at a chosen external laboratory contracted for this purpose by GSK Biologicals.

2. Cellular response to the recNY-ESO-1 + AS15 ASCI

So far, in contrast to serology, there is no standard or validated technique available to monitor the T-cell response induced by immunization with a recombinant protein in cancer patients. The techniques described in this section may be updated to the best available at the time of analysis. Dependent on the immunogenicity of the tumor-antigen targeted, the T-cell responses can either be measured directly *ex vivo* or will require a step of specific *in vitro* re-stimulation of the T-cells. The presence of specific cells ***may*** be documented by measuring the production of cytokines, by measuring a cytolytic activity (e.g. by detection of Granzyme, Perforin or CD107a marker), specific activation markers upon antigen encounter or by tetramer staining using known T cell-epitopes using appropriate assays. The percentage of those T-cells can then be determined by ELISPOT or by Fluorescence-Activated Cell Sorter (FACS), after surface and/or intracellular cytokine staining***(Amended 03 September 2014)***.

Briefly, the characterization of the T-cell response ***may*** be performed as follows***(Amended 03 September 2014)***:

- The detection and functionality of the T-cell response will be assessed by stimulating PBMCs with pools of peptides spanning the entire NY-ESO-1 protein (after *in vitro* stimulation in order to amplify specific $CD4^+$ and $CD8^+$ T-cell populations if necessary). This will be measured by FACS, including their capacity to produce cytokines and to present other markers of lytic capacity such as Perforin and Granzyme, or activation such as CD107a, CD40L or CD137.
- Depending on the *ex vivo* frequency of NY-ESO-1 specific T-cells and the availability of antigen-specific tetramers, the phenotype of cells can be analyzed in order to evaluate the nature of the specific response elicited (effector/memory). For this, expression of several surface markers such as CCR7, CD45RA, CD27 and CD28 can be measured by FACS or by another updated technology and markers.

In order to obtain a more precise picture of the patient's CMI response, tetramer assays could be carried out depending on the patient's HLA typing. Several tetramers are already available which would allow monitoring of the evolution of NY-ESO-1 specific $CD4^+$ and $CD8^+$ T-cell responses in patients after immunization.

The NY-ESO-1 cellular response will be assessed before immunization (to determine baseline values) and at several time points in each cycle of immunization (see Table 6 to Table 9).***(Amended 03 September 2014)***

7. TRANSLATIONAL RESEARCH

As of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. In case testing would be performed in the future, testing will be done in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below. (Amended 03 September 2014).

This section provides details on the translational research that may be done on the biological samples collected during this study. These different tests ***may*** only be performed on biological samples from patients who voluntarily gave their consent to these specific assessments on the ICF, unless specified as mandatory for participation in the clinical study (see Section 7.2.1). ***(Amended 03 September 2014).***

Refusal to participate in the optional translational research procedures will involve no penalty or loss of benefits to which the patient would otherwise be entitled. These tests will be performed at GSK Biologicals or a validated laboratory designated by GSK Biologicals. Based on the data generated in the study and in other studies, not all samples from patients consenting to this optional research may be processed.

7.1. Analysis of expression of NY-ESO-1 and other tumor antigens on presenting lesion and on any new lesions

The presenting lesion will also be tested for the expression of other tumor antigens such as MAGE-A3, MAGE-C2, LAGE-1, PRAME and WT1. Any new lesions will be tested for NY-ESO-1 expression and also for expression of the other tumor antigens just mentioned.

These tests will be performed on fresh tumor tissue and on formalin-fixed paraffin-embedded (FFPE) tissue (if available). The tumor samples will be processed as described in the SPM.

The tumor antigen mRNA expression levels in tumor samples will be determined using a GSK qualified assay based on quantitative RT-PCR or any updated technique at the time of the analysis. The level will be calculated relative to normalization controls. A patient will be defined as having a tumor antigen-positive tumor if the tumor antigen transcript level in the tumor is above an arbitrarily fixed cut-off level. Additional analysis of tumor antigen protein expression might be performed by using an appropriate technique such as immunohistochemistry (IHC) or any updated technology.

These tests will be performed at GSK Biologicals or at another laboratory or laboratories chosen and contracted for this task by GSK Biologicals.

7.2. Gene profiling research

7.2.1. Gene profiling on the baseline tumor lesion

The research to evaluate the possible existence of a specific predictive NY-ESO-1 gene profile that would be different from the "predictive MAGE-A3 gene profile" will be very informative and is therefore mandatory. It is worth noting that this will not require any other tumor tissue sample than the one used for the mandatory NY-ESO-1 expression screening (inclusion criteria for the study). In addition, the clinical benefit induced by the recNY-ESO-1 + AS15 ASCI will also be evaluated in the subgroup of patients who present the predictive MAGE-A3 gene signature. Moreover the incidence of expression of these two gene profiles will be evaluated in all patients who provided the ICF consent (e.g. screening failure patients). Cancer is a heterogeneous disease, including its cellularity, different genetic alterations and diverse clinical behavior patterns. Many analytical techniques have been used to study human tumors and to classify them into groups that can predict clinical behavior.

DNA microarrays have made significant contributions to this field by detecting similarities and differences amongst tumors by the simultaneous analysis of the expression of thousands of genes. DNA microarrays have allowed investigators to develop expression-based classification of tumors. Efforts aimed at discovering independent predictors of clinical outcome have identified molecular subsets of cancer based on these gene expression profiles. Subcategories of lymphoma, breast carcinoma and sarcomas with distinct prognosis and/or clinical behavior were recognized. Recently, two subgroups of metastatic melanoma lesions were identified; however, these had no predictive correlation with clinical outcome [Bittner, 2000; Wang, 2002].

Despite significant efforts to identify independent predictors of melanoma outcome, no generally accepted histopathological or molecular marker defines disease subsets with clinically different outcomes [Byers, 1998; Weyers, 1999; Busam, 2004]. But analyses of gene expression have shed new light on the progression from local to metastatic disease as well as on melanoma immune responsiveness [Carr, 2003; Wang, 2002; Haqq, 2005]. In a previous GSK Phase II study with MAGE-A3 ASCI, 75 patients with progressive, unresectable Stage III or Stage IV M1a MAGE-A3 (+) melanomas, were randomized to receive recMAGE-A3 + AS15 ASCI or recMAGE-A3 + AS02B ASCI. Gene expression profiling was performed on tumor biopsies taken prior to any immunotherapy. Two clusters of genes or gene signatures were identified that were either associated with clinical benefit - MAGE-A3 positive gene signature, or progressive disease - MAGE-A3 negative gene signature [Louahed, 2008].

Total RNA will be extracted from the fresh tumor tissue samples using an appropriate extraction kit. A fraction of the extracted RNA (e.g. 50 ng) will be used to hybridize a gene expression chip with a locked set of genes (e.g. Affymetrix or other commercial chips). Further gene expression profiling and validation of differentially expressed genes associated with clinical response might be performed by quantitative polymerase chain reaction (Q-PCR) on remaining or newly extracted total RNA from the tumor samples.

FFPE tumor tissue samples, when available, might also be used for gene expression profiling and validation of differentially expressed genes by using appropriate techniques such as Q-PCR, immunohistochemistry (IHC) or proteomic analysis.

7.2.2. Gene profiling and NY-ESO-1 expression testing on additional lesions

These tests will be performed in case the investigator plans to perform or has performed an additional tumor biopsy or a tumor resection during the study (e.g. palliative surgery, see Section 5.6.1) or because of disease progression. If the procedure occurs in the context of disease progression, the patients will be proposed to sign a separate ICF to allow a sample of this lesion to be sent to GSK Biologicals or a validated laboratory designated by GSK Biologicals for the optional research on gene profiling.

The details of this research are the same as for the gene profiling research on a fresh tissue sample from the initial tumor lesion at baseline (see Section 7.2.1).

7.3. *Natural immunity and* antigen spreading induced by the recNY-ESO-1 + AS15 ASCI *(Amended 03 September 2014)*

The aim of a cancer immunotherapeutic is to stimulate the patient's cellular and humoral immune response in order to eliminate the cancer cells. Although such immune responses can be detected upon administration of a cancer immunotherapeutic, the direct impact of this response, especially the T-cell response, on the clinical activity has not yet been demonstrated; some patients who show evidence of tumor regression upon administration of a cancer immunotherapeutic have very low T-cells directed against the target antigen of the immunotherapeutic [Lurquin, 2005]. It has now been shown that in melanoma patients who have been immunized against MAGE-A3, T-cells directed against other tumor antigens (i.e. tumor antigens not present in the immunotherapeutic) were enriched after anti-MAGE-A3 immunization [Germeau, 2005]. Moreover, these anti-tumor T-cells were found to be more frequent inside metastases than anti-MAGE-A3 T-cells, suggesting that they could have a role in the effective tumor destruction [Lurquin, 2005].

On the basis of these findings, we will assess whether administration of the recNY-ESO-1 + AS15 ASCI is able to induce antibodies and T-cells against other tumor antigens that are co-expressed by the patient's tumor (e.g. MAGE-C2, MAGE-A3, LAGE-1, WT1, PRAME) in addition to tissue differentiation or overexpressed tumor antigens such as, for instance, Melan-A/MART-1 or gp100. The expression of these tumor antigens in the tumor presenting at baseline or in any progressive lesion(s) ***may*** be assessed ***as well*** by quantitative RT-PCR or any updated technique at the time of the analysis. Validation of tumor antigen expression (including NY-ESO-1) at the protein level may also be performed by IHC or other proteomic analytical method. ***(Amended 03 September 2014).***

The analyses will be performed on fresh tissue put in *RNAlater*® immediately and used for the mandatory NY-ESO-1 expression screening (inclusion criterion for the study) or additional lesions excised during the study, and on serum and blood samples collected for

analysis of the immune responses to these other tumor antigens elicited by the recNY-ESO-1 + AS15 ASCI administrations (see Table 11).

7.4. Gene profiling of immunological response to the recNY-ESO-1 + AS15 ASCI

This will allow investigation of the patient's immunological response status prior to the start of the ASCI immunizations. This general gene expression profile will be used to identify genes that may be involved in the immunological response to the recNY-ESO-1 + AS15 ASCI. We will assess the kinetics of the immune response induced by the study treatment to get insight into the adaptive and the innate immune response that may correlate with clinical benefit.

If enough material is available, the cellular response to the recNY-ESO-1 protein will be examined upon *in vitro* re-stimulation. Total PBMCs harvested before and after ASCI administration will be stimulated twice *in vitro* with NY-ESO-1 or other cancer antigen - peptide pool. The general gene expression profile will be determined from unstimulated and stimulated PBMCs.

No additional blood sampling is needed as this gene profiling of the immune response may be performed on any of the blood samples collected for the analysis of the ***humoral or*** cellular immune response to the ASCI . Gene expression profiling will be performed on total RNA extracted from whole blood or from purified populations ($CD4^+$ T-cells, $CD8^+$ T-cells, antigen-presenting cells) from PBMC by hybridization to a gene expression chip with a locked set of genes (e.g., Affymetrix or other commercial chips) ***or by the use of epigenetic markers (e.g. methylation).*** Further gene expression profiling and validation may be performed by Q-RT-PCR. ***(Amended 03 September 2014).***

7.5. Proteomic profiling

Protein rather than mRNA regulate and mediate cellular function. Studies comparing mRNA and protein levels often showed a weak correlation between them. Thus it would be highly beneficial to have proteomic information along with gene profiling. Proteomics has been shown in the literature to be a good technique to study patients' prognosis and response to therapy [Pusztai, 2004]. There has been little proteomic information to date on the serum or tumor proteomics of patients responding or not to the ASCI.

Using ***appropriate*** techniques the serum or tumor proteome may be analyzed .This will identify useful bio-markers for responsiveness to the NY-ESO-1 antigen. ***(Amended 03 September 2014).***

This optional proteomic profiling may be performed on any of the blood samples collected for analyses of the humoral and cellular immune responses to the ASCI or on the tumor tissue sample used for the mandatory NY-ESO-1 expression test during screening, including FFPE samples.

7.6. Analysis of tumor immune infiltration

Immunotherapeutics are designed to boost tumor-specific T-cell responses in cancer patients. Nevertheless, the presence of tumor antigen-specific T-cells in peripheral blood of immunized patients does not always demonstrate a clear correlation with the clinical outcome, leaving the open question whether changes in tumor T-cell infiltration following immunotherapy could correlate better with clinical outcome. Moreover, the predictive gene signature identified in melanoma and NSCLC (refer to Section 1.3.4) indicates that the capacity of the tumor to be infiltrated by lymphocytes may be a key factor to support immunotherapy approaches.

To address the correlation of tumor T-cell infiltration before and after immunotherapy with clinical outcome, suitable markers to assess immune infiltration will be evaluated. The markers, as well as the techniques used for assessing immune infiltration may be updated at the time of analysis.

This analysis will be done using either fresh tumor tissue or FFPE tumor samples if available at screening and later biopsies collected during the study and at disease progression.

7.7. DNA promoter methylation analysis of NY-ESO-1 and other tumor antigens

Gene methylation (i.e., addition of a methyl group to a cytosine residue) is a control mechanism that regulates gene expression. Methylation of DNA occurs at CpG sites, where cytosine (C) is attached next to guanine (G) by a phosphate atom (p). Such CpG sites, located at the promoter and first exon of genes, are known as CpG islands (or CpG dinucleotide-rich region). In several diseases, these regions can be aberrantly methylated. When promoter regions are hypo-methylated, abnormally high levels of protein can be produced. Aberrant methylation of specific genes has been shown to be associated with the presence and development of some cancer types. The pattern of gene methylation in tumor cells is often specific to the tissue of origin and could be used to improve cancer detection, assess cancer aggressiveness, and predict a tumor's response to therapy [Baylin, 2006; Jones, 2005]. The correlation of mRNA expression levels of genes in the ASCI predictive gene signature or other genes that could be predictive with their methylation status will be performed for the genes for which methylation assays are available.

It has been shown that expression of cancer-testis antigens (CTAs) is re-activated during tumorigenesis and experimentally following demethylation of their promoter region [Simpson, 2005]. Even though, different studies have shown that pharmacological demethylation of tumor cell lines causes NY-ESO-1 re-expression *in vitro* [Weiser, 2001; Natsume, 2008; Woloszynska-Read, 2008], only one study has shown that the heterogeneous expression of NY-ESO-1 in ovarian tumor cells by IHC might be correlated with promoter demethylation in the NY-ESO-1-positive cell population [Woloszynska-Read, 2008]. It is then important to corroborate these findings and study their possible association with NY-ESO-1 ASCI efficacy and its implication for the development of a non-invasive screening test based on circulating tumor cells or circulating tumor cells DNA detection by NY-ESO-1 promoter methylation status.

Tumor cell DNA can be detected from the blood of melanoma patients even in early disease [Kounalakis, 2005]. This DNA can for instance originate from the primary tumor, where some cells are lyzed releasing DNA material in the blood or from circulating tumor cells. The presence of NY-ESO-1 expressing tumor cells should therefore be anticipated if the presence of demethylated NY-ESO-1 promoter DNA is detected in the patient's blood. The detection of NY-ESO-1 specific demethylated DNA from the blood could lead to the development of non-invasive methods to identify the presence of NY-ESO-1 expressing tumor cells. It could also potentially be used to anticipate the efficacy of the treatment, if the level of the circulating tumor DNA is increased over time. Practically, this approach could broaden the patient population for a treatment with NY-ESO-1 ASCI compared to the traditional methods of NY-ESO-1 detection by RT-PCR on a tumor sample.

This part of the translational research aims to analyze whether the methylation status of the NY-ESO-1 gene in tumor tissue, as assessed by using DNA from tumor tissue or blood, can be correlated with NY-ESO-1 mRNA and protein expression in the tumor.

The methylation status of regulatory regions of genes encoding other tumor antigens (e.g., MAGE-C2, PRAME, LAGE-1, MAGE-A3 and WT1) will also be assessed in the cases for which a methylation assay is available. It will be analyzed to what extent specific DNA methylation as detected in blood and tumor lesions correlates with gene expression. DNA methylation analysis will be performed based on the availability of methylation assays.

In addition, methylation status of regulatory regions of genes encoding transcriptional factors relevant in the regulation of immune response (such as *Foxp3, GATA-3, T-bet, ROR-γT* ***expressed by helper CD4 + T cells)*** might be analyzed. ***In the case of Foxp3 evaluation, the presence and characterization of circulating T regulatory cells may be evaluated in parallel or independently of the realization of these transcriptional profiling. (Amended 03 September 2014).***

A fraction of the stored serum and blood collected over time for the immunological analyses will be used for the methylation analysis. DNA methylation will also be assessed either on fresh tumor tissue or FFPE tumor sections used for NY-ESO-1 detection and additional biopsies taken during the study and at disease progression.

7.8. Pharmacogenetics analyses

Pharmacogenetics (PGx) (also referred to as pharmacogenomics) is the study of variability in response to a treatment due to hereditary factors in different individuals. There is increasing evidence that an individual's genetic composition (i.e., genotype) may impact clinical outcome to treatment with therapy, in terms of efficacy and/or safety and tolerability. Besides individual genetic variability, it has been shown that changes in DNA sequences occurring in cancer cells might also correlate with response to a given treatment [Goetz, 2007; Schroth, 2007; Higgins, 2009; Coate, 2009; Ding, 2008].

In this study, PGx will, next to the analysis of the patient's genotype, include studying whether changes in the genes of the tumoral cells might correlate with treatment response. The goal of the PGx research is thus to find genomic biomarkers that correlate with treatment efficacy, safety or tolerability. This analysis could be done at the DNA and/or RNA level.

Two types of material will therefore be used:

- For the individual genetic variability: a fraction of the stored blood collected over time for the immunological analysis (cellular immunity tests) will be used from the patients who consent to PGx research. It is recommended that the sample used for this analysis should be the one taken at Visit 1. However, any study sample collected while the patient is participating in the clinical study may be used.
- For the tumor genetic variability: this analysis might be performed on the tumor sample collected for screening, on tumor tissue samples from additional resected lesions or on tumor invaded lymph node when material is available and of appropriate quality. Of note, this investigation of the tumoral cells will not require additional material.

The specific type of genetic investigation to be applied will be dependent on the most scientifically feasible approach available to address understanding of the response of the recNY-ESO-1 + AS15 ASCI.

Generally, two approaches to explore genetic variation in response to medicine may be applied:

1. Search and analysis of candidate genes, which would have been shown or hypothesized to be associated with the effects of ASCI or that have been shown or hypothesized to play a role in melanoma development or progression. These analyses could aim at determining DNA sequence variation (mutations) in the regulatory or coding region of these genes, translocations and copy number variation using appropriate techniques that might involve whole genome interrogation.

2. Genome-wide single nucleotide polymorphisms (SNP) scanning: through the evaluation of large numbers of polymorphic markers, such as SNPs, throughout the genome, sets of markers may be identified that associate with differential response to medicine (safety and efficacy). These whole genome scans provide a hypothesis free approach, where there is limited mechanistic insight. Whole genome scans could be performed using standardized SNP arrays, for example commercially available Affymetrix or Illumina SNP chips targeting either 100,000 or 500,000 SNP coverage of the genome or any updated technology including other techniques that interrogate the whole genome. Validation of the findings might require the use of alternative approaches to detect SNPs for example genotyping PCR. These investigations will be limited to the response of study NY-ESO-1 ASCI.

DNA extracted from the blood or tumor samples may be subjected to sample quality control analysis. This analysis might involve the genotyping of several genetic markers to confirm the integrity of individual samples.

PGx analyses will be carried out at GSK Biologicals laboratories, laboratories approved by GSK Biologicals and/or associated with GSK Biologicals.

8. HEALTH ECONOMICS

Not applicable.

9. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The investigator or site staff is/are responsible during the study for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

Each patient will be instructed to contact the investigator immediately should the patient manifest any signs or symptoms they perceive as serious.

9.1. Safety definitions

9.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation patient, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of AEs to include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose *per se* should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with ASCI treatment administration.
- Significant failure of expected pharmacological or biological action.

Examples of AEs to NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- For therapeutic studies, the disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the patient's condition.

AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of patient's previous therapeutic regimen).

Example of events to be recorded in the medical history section of the eCRF:

- Pre-existing conditions or signs and/or symptoms present in a patient prior to the start of the study (i.e. prior to the first ASCI administration).

9.1.2. Definition of a serious adverse event

A serious adverse event (SAE) is any untoward medical occurrence that:

a. Results in death.

b. Is life-threatening.

NB: The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalization or prolongation of existing hospitalization.

NB: In general, hospitalization signifies that the patient has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition (known/diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity, or

NB: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect in the offspring of a study patient

f. Is a Grade 4 AE according to the Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0).

g. In addition, in this study, Grade 3 or higher pIMDs will be considered as medically significant and will therefore be notified as SAE.

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

9.1.3. Potential immune mediated diseases

Potential immune mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in the table below.

Table 12 Examples of AEs to be recorded as pIMDs (amended 3 September 2014)

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: e.g. non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy). • Narcolepsy	• Systemic lupus erythematosus and associated conditions • Systemic Scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including Dermatomyositis, Polymyositis, • Antisynthetase syndrome • Rheumatoid arthritis and associated conditions including Juvenile chronic arthritis and Still's disease) • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localised Scleroderma (Morphoea)
Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis.	• Inflammatory Bowel disease, including Crohn's disease, ulcerative colitis, microscopic colitis, ulcerative proctitis • Celiac disease • Autoimmune pancreatitis	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

Vasculitides	Blood disorders	Others
• **Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.** • **Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.**	• **Autoimmune hemolytic anemia** • **Autoimmune thrombocytopenia** • **Antiphospholipid syndrome** • **Pernicious anemia** • **Autoimmune aplastic anaemia** • **Autoimmune neutropenia** • **Autoimmune pancytopenia**	• **Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)** • **Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy)** • **Autoimmune myocarditis/cardiomyopathy** • **Sarcoidosis** • **Stevens-Johnson syndrome** • **Sjögren's syndrome** • **Idiopathic pulmonary fibrosis** • **Goodpasture syndrome** • **Raynaud's phenomenon**

However, the investigator will exercise his/her medical and scientific judgment in deciding whether other immune-mediated diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

When there is enough evidence to make *a*ny of the diagnoses listed in Table 12, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent one of these diagnoses, should be recorded and reported as AEs but not as a pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

In order to facilitate the documentation of pIMDs in the eCRF, a pIMD standard questionnaire and a list of preferred terms (PTs) and PT codes corresponding to these diagnoses will be available to the investigators at the study start.

9.1.4. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

Abnormal laboratory findings (e.g. clinical chemistry, hematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE, as defined in Section 9.1.1 or of a SAE, as defined in Section 9.1.2. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

9.2. Events or outcomes not qualifying as adverse events or serious adverse events

9.2.1. Progression of the melanoma

An event which is part of the natural course of the disease under study (i.e., disease progression), is captured as an efficacy measure. Therefore it does not need to be reported as an SAE.

Progression of the tumor will be recorded in the clinical assessments in the eCRF. Death due to progressive disease is to be recorded on a specific form in the eCRF but not as an SAE.

However, if the investigator considers that there was a causal relationship between the administration of the ASCI or protocol design/procedures and the disease progression, then this must be reported as an SAE.

Any new primary cancer (non-related to the cancer under study) must be reported as an SAE.

9.2.2. Pregnancy

Any female patients that are pregnant or lactating at the time of ASCI administration must not receive additional doses of the ASCI but may continue other study procedures at the discretion of the investigator.

The investigator, or his/her designee, will collect pregnancy information on any patient who becomes pregnant while participating in this study from the first ASCI administration ***until the Concluding Visit***. ***(Amended 03 September 2014).***

The investigator, or his/her designee, will record pregnancy information on the Pregnancy Report Form and submit it to GSK within 24 hours of learning of a patient's

pregnancy. The patient will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether that be full-term or prematurely, information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than six to eight weeks following the estimated delivery date.

While pregnancy itself is not considered an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or a SAE, as described in Sections 9.1.1 and 9.1.2, and will be followed as described in Section 9.4.4.

A spontaneous abortion is always considered to be a SAE and will be reported as described in Section 9.4. Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related in time to the receipt of the investigational product will be reported to GSK Biologicals as described in Section 9.4. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

Information on pregnancies identified during screening/prior to ASCI administration is not required to be collected and communicated to safety.

9.3. Detecting and recording adverse events, serious adverse events and pregnancies

9.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies

All AEs (except AEs categorized as pIMDs - see Section 9.1.3) starting from first dose to 30 days after the last dose of the ASCI must be recorded into the Adverse Event screen in the patient's eCRF, irrespective of intensity or whether or not they are considered ASCI administration-related.

The standard time period for collecting and recording SAEs (except SAEs categorized as pIMDs - see Section 9.1.3) will begin at the first receipt of ASCI and will end 30 days following administration of the last dose of ASCI for each patient. See Section 9.4 for instructions on reporting and recording SAEs.

The standard time period for collecting and recording severe toxicity events, pIMDs and pregnancy will begin at the first receipt of the product and last until the end of the study (Table 13).

In addition to the above-mentioned reporting requirements and in order to fulfill international reporting obligations, SAEs that are related to study participation (e.g. protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine or any fatal SAE will be collected and recorded from the time the patient consents to participate in the study until she/he is discharged.

Table 13 Reporting periods for AEs, SAEs, pIMDs and pregnancies

Study activity	Screening phase	Treatment phase			Concluding visit [1]
	ICF signature to Visit 1	Visit 1	Visit X (X= 2 to 28)	30 days after last ASCI administration	Visit 29
Reporting of AEs and SAEs					
Reporting of severe toxicity events					
Reporting of pIMDs and pregnancy					
Reporting of SAEs related to the study treatment					
Reporting of SAEs related to study participation and concurrent GSK medications, and reporting of any fatal SAEs, not due to disease progression					

1. Or the concluding visit for patients withdrawn from study treatment(s).

(Amended 03 September 2014).

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 13. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the investigator will promptly notify the Study Contact for Reporting SAEs.

9.3.2. Evaluation of adverse events and serious adverse events

9.3.2.1. Active questioning to detect adverse events and serious adverse events

As a consistent method of soliciting AEs, the patient should be asked a non-leading question such as:

'*Have you felt different in any way since receiving the ASCI or since the previous visit?*'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the eCRF screens as applicable. It is not acceptable for the investigator to send photocopies of the patient's medical records to GSK Biologicals instead of the appropriate completed AE/SAE screens in the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all patient identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

9.3.2.2. Assessment of adverse events

9.3.2.2.1. Assessment of intensity

Severity of AEs will be assessed according to the CTCAE; Version 4.0.

The investigator will assess the maximum intensity that occurred over the duration of the event for all AEs, including SAEs reported during the study. The assessment will be based on the investigator's clinical judgment.

The intensity of each AE and SAE recorded in the eCRF or SAE Report screens, as applicable, should be assigned according to the table given in the CTCAE (Version 4.0).

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets the definition in Section 9.1.2.

9.3.2.2.2. Assessment of causality

The investigator is obligated to assess the relationship between the ASCI and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. Alternative plausible causes, based on the natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the ASCI treatment will be considered and investigated. The investigator will also consult the Investigator Brochure (IB) in the determination of his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

In case of concomitant administration of multiple products, it may not be possible to determine the causal relationship of AEs to the individual product administered. The investigator should, therefore, assess whether the AE could be causally related to the ASCI administration rather than to the individual products.

Causality of all other AEs should be assessed by the investigator using the following question:

Can we eliminate the fact that the AE is unambiguously explained by a cause other than the study product (e.g. progressive tumor, accident, infection, natural history of a pre-existing disorder, etc.)?

YES	:	The AE will be considered as at least possibly related to the study product.
NO	:	The AE is not related to the study product. There are other, more likely causes and the study product is not suspected to have contributed to the AE.

Non-serious and serious AEs will be evaluated as two distinct events. If an event meets criteria to be determined ‘serious’ (see Section 9.1.2 for definition of serious adverse event), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors applicable to each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the ASCI, if applicable.
- Erroneous administration.
- Other cause (specify).

9.3.2.3. Assessment of outcomes

Outcome of any non-serious AE occurring within 30 days post-ASCI administration or any SAE reported during the entire study will be assessed as:

- Recovered/resolved.
- Not recovered/not resolved.
- Recovering/resolving.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

9.4. Reporting and follow-up of adverse events, serious adverse events and pregnancies

9.4.1. Prompt reporting of serious adverse events and other events to GSK Biologicals

SAEs will be reported promptly to GSK as described in Table 14 once the investigator determines that the event meets the protocol definition of an SAE.

Pregnancies will be reported promptly to GSK as described in Table 14 once the investigator becomes aware of a pregnancy in the time period defined in Section 9.3. The patient will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether that be full-term or premature, information on the status of the mother and child will be forwarded to GSK. Generally, follow-up should be no longer than 6 to 8 weeks following the estimated delivery date.

Table 14 Time frames for submitting SAEs and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up Information on a Previous Report	
	Time Frame	Documents	Time Frame	Documents
All SAEs	24 hours*	SAE screen	24 hours*	SAE screen
Severe toxicities	24 hours*	eCRF	24 hours	eCRF
pIMDs	24 hours	SAE screen	24 hours	SAE screen
Pregnancy	24 hours*	Pregnancy Report Form	24 hours*	Pregnancy Report Form

* Time frame allowed after receipt or awareness of the information.

In case the electronic reporting system is temporarily unavailable, a back-up system is in place. Please refer to Section 9.4.3 for a detailed description. Please see the Sponsor Information Sheet for contact details.

Back-up Study Contact for Reporting SAEs and severe toxicities
GSK Biologicals Clinical Safety & Pharmacovigilance Fax: +PPD or +PPD **24/24 hour and 7/7 day availability**

9.4.2. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK Biologicals in accordance with the procedures detailed in Section 9.4.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authorities and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other patients are met.

Investigator safety reports are prepared according to the current GSK Biologicals policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the investigational product and unexpected. The purpose of the report is to fulfill specific regulatory and Good Clinical Practice (GCP) requirements, regarding the product under investigation.

9.4.3. Completion and transmission of SAEs reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study patient, the investigator will complete and submit the information in the SAE screens in eCRF within 24 hours. The SAE screens in eCRF will always be completed as thoroughly as possible with all available details of the event and will be submitted by the investigator. If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying GSK Biologicals of the event and completing the SAE screens in eCRF. The SAE screens in eCRF should be updated when additional relevant information is received WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

Upon notification, GSK Biologicals will in turn inform all investigators by fax or email (within 24 hours) about any SAE considered as related or possibly related to the ASCI administration. The investigators will acknowledge receipt of this information.

Any severe toxicity event must be entered in the patient's eCRF within 24 hours of the event becoming known to the investigator.

9.4.3.1. Back-up system in case the electronic SAE reporting system does not work

If the SAE reporting system has been down for 24 hours, the investigator or his/her delegate should fax an SAE report form directly to the GSK Biologicals Central Safety department (please refer to Section 9.4.1) within 24 hours. The maximum timeline for reporting SAEs to central safety is therefore 48 hours.

NB. This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow.

As soon as the electronic reporting system is working again, the investigator or delegate must update the SAE screens in the eCRF within 24 hours.

The final valid information for regulatory reporting will be the information reported through the electronic system.

When additional information is received on a SAE after freezing of the patient's eCRF, new or updated information is to be recorded on the paper SAE Report Form, with all changes signed and dated by the investigator. The updated SAE Report Form should be resent to GSK Biologicals WITHIN 24 HOURS of receipt of the follow-up information.

9.4.3.2. Back-up system in case the electronic system for reporting of severe toxicities does not work

If the electronic system for severe toxicity reporting has been down for 24 hours, the investigator or his/her delegate should fax a severe toxicity form directly to the GSK Biologicals Central Safety Department (please refer to Section 9.4.1) within 24 hours. The maximum timeline for reporting severe toxicities is therefore 48 hours.

As soon as the electronic reporting system is working again, the investigator or delegate must update the severe toxicity screen in the eCRF within 24 hours.

The final valid information for regulatory reporting will be the information reported through the electronic system.

9.4.4. Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a patient is pregnant, the investigator (or designate) must complete a Pregnancy Report Form and fax it to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) WITHIN 24 HOURS.

The Pregnancy Report Form will always be completed as thoroughly as possible with all available details and then dated and signed by the investigator (or designate). Even if the investigator does not have all information regarding the pregnancy, the form should still be completed and forwarded to GSK within 24 hours. Once additional relevant information is received, the form will be updated and forwarded to GSK WITHIN 24 HOURS.

In absence/dysfunction of facsimile equipment, the Study Contact for Reporting SAEs should be notified by telephone within 24 hours. As soon as the facsimile equipment is working again, the investigator (or designate) must fax the Pregnancy Report Form to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) within 24 hours.

9.4.5. Reporting of pIMDs to GSK Biologicals

Once onset of a new pIMD or exacerbation of a pre-existing pIMD is diagnosed (serious or non-serious) in a study patient, the investigator (or designate) must complete the information in the SAE screens of the eCRF WITHIN 24 HOURS after he/she becomes aware of the diagnosis. A field on the SAE screen allows to specify that the event is a pIMD and whether it is serious or non serious. The SAE screens will always be completed as thoroughly as possible with all available details of the event, in accordance with the pIMD standard questionnaire provided. Even if the investigator does not have all information regarding a pIMD, the SAE screens should still be completed within 24 hours. Once additional relevant information is received, the SAE screens in the eCRF should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

Refer to Section 9.4.3.1 for back-up system and updating of SAE information after freezing of the patient's eCRF.

9.4.6. Follow-up of adverse events and serious adverse events

After the initial AE/SAE report, the investigator is required to proactively follow each patient and provide further information to GSK Biologicals on the patient's condition.

All SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the end of the study.

All AEs and pIMDs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last ASCI administration.

All severe toxicities and pIMDs documented at a previous visit/contact and designated as not recovered/not resolved or recovering / resolving will be reviewed at subsequent visits/contacts until the end of the study.

Investigators will follow-up patients:

- With SAEs or patients withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the patient is lost to follow-up.
- Or, in the case of other non-serious AEs and pIMDs until they complete the study or they are lost to follow-up.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relative to the subsequent course of such abnormalities noted for any patient must be made available to the Site Monitor.

GSK Biologicals may request that the investigator perform or arrange for the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a patient dies during participation in the study or during a recognized follow-up period, GSK Biologicals will be provided with a copy of any available post-mortem findings, including histopathology.

9.5. Treatment of adverse events

Treatment of any adverse event is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the patient's eCRF. Refer to Section 9.4.

9.6. Unblinding

Not applicable.

9.7. Emergency unblinding

Not applicable.

9.8. Patient card

Study patients must be provided with the address and telephone number of the main contact for information about the trial.

Investigator/delegate should therefore provide a "patient card" to each patient. The aim of this card is to inform any physician having to deal with a patient in an emergency situation that the patient is in a clinical trial and that he/she can contact the trial investigator for more relevant information.

Patients must be instructed to keep these cards in their possession at all times.

10. PATIENT COMPLETION AND WITHDRAWAL

10.1. Patient completion

10.1.1. Patient completion of ASCI administration

A patient will be considered to have completed the study treatment when he/she has received injection number 24 with the ASCI.

Administration of the ASCI should continue until documented disease progression (except for SPD (Section 6.6.6)), or withdrawal due to contraindications for subsequent ASCI administration (see Section 5.5.2).

Once the patient has been withdrawn from the ASCI, the reason must be documented in the patient's medical records and eCRF.

10.1.2. Patient completion of study

A patient will be considered to have completed the study when he/she has reached the end of all scheduled study visits.

Once the patient has been withdrawn from the study, the reason must be documented in the patient's medical records and eCRF. ***(Amended 03 September 2014).***

10.2. Patient withdrawal

Patients who are withdrawn because of AEs must be clearly distinguished from patients who are withdrawn for other reasons. Investigators will follow patients who are withdrawn as result of a SAE/AE until resolution of the event (see Section 9.4).

Once the patient has been withdrawn, the reason must be documented in the patient's medical records and eCRF. Withdrawals will not be replaced.

10.2.1. Patient withdrawal from ASCI administration

A 'withdrawal' from the ASCI refers to any patient who does not receive the complete treatment, i.e. when no further planned dose is administered from the date of withdrawal. A patient withdrawn from the ASCI may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed, if planned in the study protocol.

The investigator will document whether the decision to discontinue further ASCI administration was made by the patient themselves, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- (Serious) adverse event (including concurrent illness, unacceptable toxicity),
- Disease progression/recurrence,
- Consent withdrawal
- Protocol violation,
- Other (specify).

In this case, a concluding visit will be performed with procedures described in Section 6.5.4. ***(Amended 03 September 2014).***

If the patient refuses or cannot undergo these study procedures, he/she will be considered as withdrawn from study (Section 10.2.2). Patients who withdraw, or are withdrawn, from the study will not be replaced.

10.2.2. Patient withdrawal from the study

A patient will be considered as being withdrawn from the study when he/she does not undergo any further planned study visit after the date of withdrawal.

A patient may voluntarily discontinue participation in this study at any time. The investigator may also, at his/her discretion, discontinue the patient from participating in this study at any time. In addition, if the sponsor decides to discontinue the study, no further study procedures (including administration of the ASCI) will occur.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw from the study was made by the patient or the investigator and which of the following reason applies:

- Death (any cause).
- (Serious) adverse event (including intercurrent illness, unacceptable toxicity).
- Disease progression/recurrence.
- Consent withdrawal, not due to an adverse event.
- Lost to follow-up.
- Protocol violation.
- Moved from study area.
- Other (specify).

All data collected until the date of withdrawal/last contact of the patient will be used for the analysis.

10.3. Screen and baseline failures

A patient is considered to be a screen or baseline failure if he/she signs the ICF, but withdraws before receiving the first dose of ASCI treatment.

All patients having given consent to undergo NY-ESO-1 expression screening upon resection of their tumor will be referenced into a screening section of the eCRF.

In addition to NY-ESO-1 expression screening, translational research will be performed on the tumor tissue sample of patients having given their specific consent for this research. For details on the translational research, refer to Section 7.

11. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

This section describes the criteria for evaluation of objectives as initially planned. However, due to the Protocol Amendment 3, not all analyses described in this section will be performed. A detailed description of the analyses that will be done will be given in the Statistical Analysis Plan. The final analysis will occur when all patients have performed their Concluding Visit or have withdrawn from the study. (Amended 03 September 2014)

11.1. Co-primary endpoint

11.1.1. Safety endpoint

- Occurrence of severe toxicities during the study treatment phase and follow-up.

Defined according to CTCAE (Version 4.0) as follows:

1. An ASCI-related or possibly ASCI-related Grade 3 or higher toxicity. Grade 3 myalgia, arthralgia, headache, fever, rigors/chills and fatigue (including lethargy, malaise and asthenia) should persist for 48 hours despite therapy in order to be taken into account.
2. An ASCI-related or possibly ASCI-related Grade 2 or higher allergic reaction occurring within 24 hours following the ASCI administration.

Note: Any severe toxicity event must be entered in the patient's eCRF within 24 hours of the event becoming known to the investigator.

11.1.2. Clinical activity endpoint

- The induction of objective clinical response (CR or PR) in the overall population.

Defined in Section 6.6.

11.2. Secondary endpoints

11.2.1. Clinical activity endpoints

The secondary clinical activity endpoints will include:

1. Occurrence of objective clinical response (CR or PR) in the population of patients who present the predictive MAGE-A3 gene signature.
2. In the overall population and in the population of patients presenting the predictive MAGE-A3 gene signature:
    - Occurrence of stable disease (SD).

- Occurrence of mixed response (MR).
- Time to Treatment Failure (TTF).

TTF is defined as the time from first treatment until the date of the last treatment administration, irrespective of the reason for study treatment discontinuation.

- Progression-free survival (PFS).

PFS is defined as the time from first treatment to either the date of ***first*** disease progression ***(PD or SPD)*** or the date of death (for whatever reason), whichever comes first. Patients alive and without disease progression are censored at the date of the last visit/contact. ***(Amended 03 September 2014)***

- Overall survival (OS).

OS is defined as the time from first treatment until death. Patients alive at the time of analysis are censored at the time of the last visit/contact.

- The duration of response for patients with CR, PR or SD status.

The duration of the response is defined as time from first objective response or SD evaluation to first PD assessment ***or death***. ***(Amended 03 September 2014).***

11.2.2. Safety endpoints

The secondary safety endpoints will include:

- Occurrence of AEs and SAEs during the study treatment period and ending 30 days after the last study treatment administration.

Note: The occurrence of SAEs (possibly) related to the recNY-ESO-1 + AS15 ASCI will be captured during the entire study duration, as described in Table 13.

11.2.3. Immunogenicity endpoints

- Immunogenicity of the NY-ESO-1 ASCI treatment will be evaluated at different timepoints.

This is explained in Section 6 and is on the basis of:

1. The anti-NY-ESO-1 humoral antibody concentration and response. ***(Amended 03 September 2014)***
2. The anti-NY-ESO-1 ***specific*** cellular (T-cell) response. ***(Amended 03 September 2014)***

11.3. Estimated sample size

The study will require a planned sample size of 34 patients having received at least one ASCI injection.

- Alpha: 0.10
- Beta: 0.10
- Levels: P0 = 0.05, P1 = 0.20, 1 – T0 = 0.75, 1 – T1 = 0.95

11.3.1. Definitions

- P0 is the unacceptable response probability which, if true, implies that the therapeutic activity does not warrant further investigation of the regimen. In the present trial, P0 has been taken as 5%.
- P1 is the lowest acceptable response probability which, if true, implies that the therapeutic activity does warrant further investigation of the regimen, provided that the incidence of severe toxicity is acceptable. In the present trial, P1 has been taken as 20%.
- (1 – T0) is the unacceptable non-severe toxicity probability which, if true, implies that the regimen does not warrant further investigation. In the present trial, (1 – T0) has been taken as 75%.
- (1 – T1) is the acceptable non-severe toxicity probability which, if true, implies that the regimen does warrant further investigation, provided that the response rate is acceptable. In the present trial, (1 – T1) has been taken as 95%.

11.3.2. Statistical errors will be

- Beta is the probability of rejecting from further trials a regimen with a true response rate at least equal to P1 and a true non-severe toxicity rate equal to or higher than (1 - T1). In the present trial, beta has been taken as 0.10.
- Alpha is the accepted probability of recommending for further investigation a regimen with a true response rate lower than P0. It is also the accepted probability of recommending for further trials a regimen with a true non-severe toxicity rate lower than (1 – T0). In the present trial, the two alphas have been taken as 0.10.
- A total of 34 patients will be enrolled and assessed for overall response and severe toxicity.

11.3.3. Study success criteria

- If ≥ 4 (4/34 = 11.8%) objective clinical responses are observed, then the treatment regimen will be considered as active.
- If ≤ 4 (4/34 = 11.8%) severe toxicities (see Section 11.1.1) are observed, then the treatment regimen will be considered as feasible from a safety point of view.

If these two conditions are met, the conclusion will be that the given regimen is active and feasible, and should be investigated further in this patient population.

If ≤ 1 (1/34 = 3%) clinical responses are observed or if ≥ 5 patients (5/34 = 14.7%) have severe toxicity, the conclusion will be that the recNY-ESO-1 + AS15 ASCI is not active enough or is too toxic and that it should not be investigated further in this patient population.

If 2 or 3 clinical responses are observed and ≤ 4 patients have severe toxicity, the study will be unconclusive (neither success nor failure).

Simulations (5000 repetitions, binomial distribution for the risk that one patient does develop a severe toxicity and for the objective clinical response) have been used to determine the percentage of trials that satisfy the criteria for further development, fail or be unconclusive, considering the different criteria defined above, and assuming different values for the severe toxicity and the clinical response probabilities on a patient level (Table 15).

For example, if the true clinical response rate is 20% and the true toxicity rate 2.5%, we have 93% probability to conclude on a success, 0.5% probability to conclude on a failure and 6.4% probability where more investigation will be needed before final conclusion; if the true clinical response rate is 5% and the true toxicity rate 20%, we have 1.5%, 91% and 7.3% probabilities, respectively.

Table 15 Outcome of simulations with both safety and clinical response

True Toxicity rate	True Clinical response rate [1]											
	5%			10%			15%			20%		
	F	U	S	F	U	S	F	U	S	F	U	S
2.5%	49%	42%	9.0%	13%	42%	45%	3.1%	20%	77%	0.5%	6.4%	93%
5.0%	50%	41%	9.0%	15%	41%	44%	5.3%	19%	75%	3.0%	6.4%	91%
10.0%	61%	32%	6.5%	35%	32%	33%	27%	15%	58%	25%	5.0%	70%
20.0%	91%	7.3%	1.5%	86%	6.6%	7.7%	84%	3.3%	12%	84%	1.1%	15%

1. F = % failure, U = % inconclusive, S = % success

11.4. Study cohorts to be evaluated

11.4.1. Total treated cohort

The total treated cohort will include all the enrolled patients who have received at least one dose of the ASCI.

11.4.2. According-to-protocol (ATP) cohort for analysis of immunogenicity

The ATP cohort for analysis of immunogenicity will include all evaluable patients (i.e., those meeting all eligibility criteria, who did not report major protocol deviation, who have complied with all the procedures defined in the protocol, who have received at least the first four study treatment administrations and have provided a result for immunogenicity evaluation within the 4 weeks following dose no. 4 and completed Visit 5). For each non-compliant patient, all data collected after protocol violation (i.e. treatment administered or blood sample collected too early or too late, concomitant medication, medical condition,…) will be eliminated from the ATP immunogenicity analyses. ***(Amended 03 September 2014)***

11.5. Derived and transformed data

11.5.1. Humoral immunogenicity

1. The cut-off value of the Enzyme-Linked Immunosorbent Assay (ELISA) assay will be defined by the laboratory before the analysis.
2. A seropositive patient is a patient whose anti-NY-ESO-1 antibody titer is higher than or equal to the cut-off value. ***(Amended 03 September 2014)***
3. Seroconversion in a patient is defined by the increase in antibodies (anti-NY-ESO-1) from a titer below the cut-off level before the treatment to a titer above the cut-off level following treatment. ***(Amended 03 September 2014)***
4. An ASCI humoral response is defined as:
    - For an initially seronegative patient: an increase in the anti-NY-ESO-1 antibody titer to above the cut-off level (i.e seroconversion).
    - For an initially seropositive patient: an increase in anti-NY-ESO-1 antibody titer to a level at least two times higher than the pre-treatment titer.
5. The geometric mean titer (GMT) is calculated by taking the anti-logarithm of the mean of the log 10 transformations. Antibody titers below the cut-off of the assay will be given an arbitrary value of half of the cut-off for the purpose of the calculation.
6. For a given patient and given immunogenicity measurement, results of missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude patients with missing or non-evaluable measurements.

11.5.2. Cellular Immunogenicity

A patient will be considered as a cellular-mediated immune responder if there is an increased amount of NY-ESO-1-specific T-cells after immunization as compared to the patient's baseline value

The cut-off above which a patient will be considered ***to present NY-ESO-1 cellular immunogenicity*** will be determined ***and specified in the Statistical Analysis plan.***

(Amended 03 September 2014)

11.5.3. Safety

For analysis of serious adverse events or adverse events by primary MedDRA term, all patients treated will be considered. Patients who did not report an event will be considered as patients without the event.

11.6. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the ***statistical analysis plan and/or*** final study report. ***(Amended 03 September 2014)***

11.6.1. Sequence of analyses

Safety data will be reviewed according to the safety rules (Section 6.3.3) and the DSMC charter, by the DSMC and GSK Biologicals.

The final analysis will be performed when all patients have done their concluding visit or have withdrawn from the study. Due to Protocol Amendment 3, not all analyses described in this section will be performed. A detailed description of the analyses that will be done will be given in the statistical analysis plan.. A full clinical study report will be written including individual data listings.

(Amended 03 September 2014)

11.6.2. Statistical considerations for interim analyses

Not applicable.

11.7. Statistical methods

All statistical analyses will be performed using SAS software and/or StatXact.

Due to Protocol Amendment 3, not all analyses described in this section will be performed. A detailed description of the analyses that will be done will be given in the statistical analysis plan. (Amended 03 September 2014)

11.7.1. Analysis of demographics/baseline characteristics

Demographic characteristics (age, gender, race, etc.) and baseline tumor characteristics such as stage or gene expression will be tabulated and analyzed by appropriate descriptive statistics:

- Frequency tables will be generated for categorical variable such as gender.
- Mean, median, standard error will be provided for continuous data such as age.
- The total number of ASCI doses administered to each patient and the length of follow-up will be summarized.

These descriptive analyses will be performed on the total treated cohort.

11.7.2. Analysis of clinical activity

- Disease progression, including death, during the study will be described in detail by patient narratives.
- Estimates of the rates of best clinical response and their 95% confidence interval (CI) will be reported.
- Kaplan-Meier curves will be estimated for TTF, PFS and OS. If study treatment failure does not occur before the patient's last study visit, then the time to study treatment failure will be censored at the date of the last visit. If progression does not occur before the patient's last study visit, then the time to progression will be censored at the date of the last visit. If the patient is withdrawn from the study treatment for other reasons than progression, then the time to study treatment failure or progression will be censored at the date of the last assessment of the patient. ***For OS, patients who are still alive at the time of the analysis will be censored at the date last known to be alive(Amended 03 September 2014)***
- In addition, other exploratory analyses may be performed if necessary.

The analysis of clinical activity will be performed on the total treated cohort.

11.7.3. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for immunogenicity. Humoral and cellular responses will also be analyzed for the total treated cohort if more than 3 patients are excluded from the ATP cohort for immunogenicity.

For each cohort, at each blood sampling time point for which results are available:

- ***Anti-NY-ESO-1 antibody s***eropositivity rate, humoral response rate and antibody geometric mean titers (GMTs) with 95% CIs ***will be measured or calculated***;
- Percentages of patients ***presenting NY-ESO-1 immunogenicity scores above cut-off*** and cellular T-cell ***responses to NY-ESO-1*** with exact 95% CIs will be given***. Estimations of the cellular T cell precursor frequencies will be given if available.***
- In addition, individual data over time will be displayed graphically

(Amended 03 September 2014).

11.7.4. Analysis of safety

The verbatim reports of AEs will be reviewed by a physician and coded according to the MedDRA Dictionary for Adverse Reaction Terminology.

All patients who received at least one ASCI injection will be included in overall toxicity analyses (total treated cohort). Patients who have discontinued treatment because of toxicity will always be included in the toxicity analyses. Toxicity and AEs occurring in ineligible patients will be reported separately.

A summary of AEs by maximum grade and cohort will be displayed. The same tabulation will be presented for events with a suspected relationship to the study treatment. A summary of AEs by maximum grade and cohort will also be provided separately for the four treatment cycles.

SAEs will be described in detail by patient narratives.

Non-hematological, acute side effects will be assessed and reported separately for each ASCI administration, and graded according to the CTCAE version 4.0 criteria.

12. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications must be fulfilled.

12.1. Remote data entry instructions

Prior to screening and/or enrolling the first potential participant, the investigator will provide the Site Monitor with a list (Site Staff Signature Sheet (SSSS)) showing the name and title, signature and initials of all site staff who have a role in the study and to whom the investigator has delegated significant study related duties such as entering data in the eCRF or changing entries in the eCRFs. If the authorized individuals change during the study, the investigator is to inform GSK Biologicals' representative of the specific change(s). The SSSS needs to be updated accordingly in order to reflect the current situation at all times.

Remote Data Entry (RDE), a validated computer application, will be used as the method for data collection.

In all cases, patient initials will not be collected nor transmitted to GSK. Patient data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

The patient information will be entered into a computer at the investigational site preferably within 5 working days of becoming available. The site will be able to modify the data to assure accuracy with source documentation. All new/updated information will be reviewed and verified by a GSK Biologicals' Site Monitor. This information will finally be stored in a central database maintained by GSK Biologicals. At the conclusion of the study, GSK Biologicals will archive the study data in accordance with internal procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the clinical study report is complete and approved by all parties.

12.2. Regulatory and ethical considerations, including the informed consent process

GSK will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable country-specific regulatory requirements, prior to a site initiating the study in that country.

The study will be conducted in accordance with all applicable regulatory requirements, including a United States Investigational New Drug Application (US IND).

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable patient privacy requirements and the guiding principles of the Declaration of Helsinki.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favorable opinion/approval of study protocol and any subsequent amendments.
- Patient informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written informed consent must be obtained from each patient prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Those sections marked as mandatory must not be modified. Clinical judgment, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

12.3. Data Safety Monitoring Committee

A Data and Safety Monitoring Committee (DSMC) composed of an independent group of experts to advise GSK Biologicals and the study investigators will be established by GSK Biologicals, and the DSMC will supervise this Phase I study in metastatic melanoma.

The Committee will review the clinical relevance of each severe toxicity event and its relationship to the ASCI administration. The DSMC should provide immediate feedback within 3 working days. For each severe toxicity event, GSK will not wait for the feedback of the DSMC to continue the dosing of the other patients.

In addition, the DSMC will receive a safety report from GSK Biologicals every 6 months (or more frequently, if necessary) for review and will review progress and safety results, and will also make recommendations to GSK Biologicals as detailed below. *Ad hoc* meetings can be convened on the basis of requests from GSK Biologicals or from any member of the DSMC. No member of the Committee may be an investigator in the study or an employee of GSK Biologicals. The operating rules of the Committee will be established jointly by the Committee and GSK Biologicals within the framework set out in the protocol. A detailed statement of the tasks of the DSMC will be given in a separate document to be prepared by GSK Biologicals in consultation with the Committee's members.

The primary responsibilities of the DSMC will be as follows:

- The DSMC will review the study progress reports provided by GSK Biologicals on a regular basis, with a view to identifying any findings that could have an effect on the conduct or validity of the present study or on the safety of the patients. If safety concerns are identified, then GSK Biologicals, the Principal Investigator(s), the DSMC and/or the applicable regulatory agencies may recommend that the study be suspended, amended, or terminated.
- The DSMC will assess the clinical relevance of each severe toxicity event and must provide feedback within 3 working days.
- On the basis of safety information, the Committee will make recommendations to the sponsor concerning the continuation, modification or termination of the trial. As soon as one of the criteria for stopping or suspending the entire study (see Section 6.3.3) is met, the DSMC will be contacted for advice as to whether to continue the study or not.

Although the DSMC will advise GSK Biologicals, the authority to suspend, amend or terminate the study remains with GSK Biologicals.

Members will be available for expert consultation on any other medical, scientific or safety issues that may arise during the trial.

12.4. GSK Biologicals' Safety Review Team

The GSK Biologicals' Safety Review Team (core members include Clinical Development Manager, Safety Product Specialist and Biostatistician) will on a regular basis review all available safety data related to the investigational product to identify any potential safety issues or signals to evaluate and to agree on action plans, if necessary.

12.5. Monitoring by or on behalf of GSK Biologicals

Monitoring visits by a formal representative of the Sponsor or a GSK Site Monitor are for the purpose of confirming that GSK Biologicals' sponsored studies are being conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and that are consistent with Good Clinical Practice (GCP) and the applicable regulatory requirement(s) (verifying continuing compliance with the protocol, amendment(s), reviewing the investigational product accountability records, verifying that the site staff and facilities continue to be adequate to conduct the study).

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a RDE review and a Source Document Verification (SDV). By SDV we understand verifying RDE entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the RDE. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the RDE will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For RDE, the monitor will mark completed and approved screens at each visit.

In accordance with applicable regulations, GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the eCRF entries will serve as the source document.

GSK will monitor the study to verify that, amongst others, the:

- Data are authentic, accurate, and complete.
- Safety and rights of patients are being protected.
- Study is conducted in accordance with the currently approved protocol and any amendments, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

12.6. Archiving of data at study sites

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. electronic for studies with an eCRF); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by ICH GCP, any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to, the following: archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

12.7. Audits

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

12.8. Ownership, confidentiality and publication

12.8.1. Ownership

All information provided by GSK and all data and information generated by the site as part of the study (other than a patient's medical records) are the sole property of GSK.

All rights, title, and interests in any inventions, know-how or other intellectual or industrial property rights which are conceived or reduced to practice by site staff during the course of or as a result of the study are the sole property of GSK, and are hereby assigned to GSK.

If a written contract for the conduct of the study which includes ownership provisions inconsistent with this statement is executed between GSK and the study site, that contract's ownership provisions shall apply rather than this statement.

12.8.2. Confidentiality

Documented evidence that a potential investigator is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

All information provided by GSK and all data and information generated by the site as part of the study (other than a patient's medical records) will be kept confidential by the investigator and other site staff. This information and data will not be used by the investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (i) information which becomes publicly available through no fault of the investigator or site staff; (ii) information which it is necessary to disclose in confidence to an IEC or IRB solely for the evaluation of the study; (iii) information which it is necessary to disclose in order to provide appropriate medical care to a study patient; or (iv) study results which may be published as described in the next paragraph. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

12.8.3. Publication

For multicenter studies, the first publication or disclosure of study results shall be a complete, joint multicenter publication or disclosure coordinated by GSK. Thereafter, any secondary publications will reference the original publication(s).

Prior to submitting for publication, presentation, use for instructional purposes, or otherwise disclosing the study results generated by the site (collectively, a 'Publication'), the investigator shall provide GSK with a copy of the proposed Publication and allow GSK a period to review the proposed Publication (at least twenty-one working days, or at least fifteen working days for abstracts/posters/presentations). Proposed Publications shall not include either GSK confidential information other than the study results or personal data on any patient, such as name or initials.

At GSK's request, the submission or other disclosure of a proposed Publication will be delayed a sufficient time to allow GSK to seek patent or similar protection of any inventions, know-how or other intellectual or industrial property rights disclosed in the proposed Publication.

If a written contract for the conduct of the study, which includes publication provisions inconsistent with this statement is executed, that contract's publication provisions shall apply rather than this statement.

13. COUNTRY SPECIFIC REQUIREMENTS

13.1. French administrative considerations

This Section includes all the requirements of the French law (n° 2004-806 of 09 August 2004), and identifies, item by item, the mandatory modifications or additional information to the study protocol.

- Concerning the "Study Population"

  In line with the local regulatory requirements, the following text in section "OTHER STUDY ELIGIBILITY CRITERIA CONSIDERATIONS" is added:

  A patient will be eligible for inclusion in this study if he /she is either affiliated to or beneficiary of a social security category.

  It is the investigator's responsibility to ensure and to document (in source document - patient notes) that the patient is either affiliated to or beneficiary of a social security category.

- Concerning the "Data Evaluation: Criteria for Evaluation of Objectives"
  Section on the "ESTIMATED SAMPLE SIZE"

  The following text concerning the "sample size assumption" is added:

  The expected number of patients to be recruited in France is declared to the French Regulatory Authority.

- Concerning the "Conduct of the Study"
  Section "REGULATORY AND ETHICAL CONSIDERATIONS, INCLUDING THE INFORMED CONSENT PROCESS"

  The following text concerning "the process for informing the patient or his/her legally authorized representative" is added:

  French Patient ICF is a document in triplicate which summarizes the main features of the study and allows collection of the patient's written consent. It also contains a reference to the authorization of ANSM and the approval from the French Ethics Committee and the maintenance of confidentiality of the returned consent form by GSK France.

In addition, the following text concerning “the process for obtaining the patient's informed consent” is added:

- When biomedical research is carried out on a minor / on an adult in the care of a “tutelle” guardian, consent is given by their legal representative and, if the committee mentioned in article L. 1123-1 considers that the research in question, because of the gravity of the restraints or the specificity of the medical acts involved, entails a serious risk of affecting their private life or the integrity of their body, by the family council if it has been instated, or by the judge of “tutelle” guardians.
- When biomedical research is carried out on an adult in the care of a “curatelle” guardian, consent is given by the patient assisted by his guardian. However, if the adult in the care of a “curatelle” guardian is invited to participate in research which the committee mentioned in article L. 1123-1 considers, because of the gravity of the restraints or the specificity of the medical acts involved, to entail a serious risk of affecting their private life or the integrity of their body, the matter is submitted to the judge of guardians who decides whether the adult is capable of giving his consent. In the case of incapacity, the judge will decide whether or not to authorize the biomedical research.
- When biomedical research, which complies with the conditions laid down in article L. 1121-8, is considered for an adult incapable of expressing his consent and not under a legal protection order, consent is given by a person of confidence as defined in article L. 1111-6 and, failing this, by a person who maintains close and stable links with the patient. However, if the committee mentioned in article L. 1123-1 considers that the research in question, because of the gravity of the restraints or the specificity of the medical acts involved, entails a serious risk of affecting their private life or the integrity of their body, consent is given by the judge of guardians.

- Finally, concerning “the management of the Patient ICFs” the following text is added:

  The first copy of the Patient ICF is kept by the investigator. The second copy is kept by the Director of the Medical Department of GlaxoSmithKline France and the last copy is given to the patient or his/her legally authorized representative.

  The second copy of all the consent forms will be collected by the investigator at the end of the trial under the Clinical Research Assistant’s (CRA's) control, and placed in a sealed envelope bearing only:

  - The study number,
  - The identification of the Centre: name of the principal investigator and number of centre),
  - The number of informed consents,
  - The date and,
  - The principal investigator’s signature.

Then, the CRA hands the sealed envelope over to the Director of the Medical Department, for confidential recording, under his responsibility.

- In section concerning the " DEMOGRAPHIC DATA " the following text is added:

  In accordance with the data-processing and freedom French law dated on 6th of January 1978 modified on the 6th of August 2004 - article 8, the ethnic origin can only be collected if the collection of this data is justified within the framework of this study.

- In section concerning the " TESTING OF BIOLOGICAL SAMPLES " the following text is added:

  In accordance with Article L1211-2 of the French Public Health Code, a biological sample without identified purpose at the time of the sample and patient's preliminary information is not authorized.

- Concerning "Administrative matters"

  The following text concerning "notification to the hospital director" is added (if applicable):

  In accordance with Article L1123-13 of the Public Health Code, the Hospital Director is informed of the commitment to the trial in his establishment. The Hospital Director is supplied with the protocol and any information needed for the financial disposition, the name of the investigator(s), the number of sites involved in his establishment and the estimated time schedule of the trial (R.1123-63).

  The following text concerning "information to the hospital pharmacist" is added (if applicable):

  In accordance with Article R.1123-64 of the Public Health Code, the Hospital Pharmacist is informed of the commitment to the trial in his establishment. The Pharmacist is supplied with a copy of the protocol (which allows him to dispense the drug(s) of the trial according to the trial methodology), all information concerning the product(s) of the trial (e.g. included in the Clinical Investigator's Brochure), the name of the investigator(s), the number of sites involved in his establishment and the estimated time schedule of the trial.

  Finally, the following text concerning "data management" is added:

  Within the framework of this clinical trial, data regarding the identity of the investigators and/or co-investigators and/or the pharmacist if applicable, involved in this clinical trial, and data regarding the patients recruited in this clinical trial (patient number, treatment number, patient status with respect to the clinical trial, dates of visit, medical data) will be collected and computerized in GSK Biologicals data bases by Laboratoire GlaxoSmithKline or on its behalf, for reasons of follow up, clinical trial management and using the results of said clinical trial. According to the Act no. 78-17 of 6th January 1978 further modified, each of these people aforesaid

has a right of access, correction and opposition on their own data through Laboratoire GlaxoSmithKline (Clinical Operations Department).

13.2. Germany specific requirements

Concerning the HIV test

Upon request from Paul-Ehrlich-Institut (Germany), HIV test is mandatory to assess the HIV status (positive - negative) of patients in Germany only. Testing will be performed during the screening phase of the study and according to local standard practice. Patients with known HIV-positive status or positive for HIV as per testing will not be eligible for entry in the study. Patients for which the test is positive will be managed according to standard local clinical practice. Patients with known HIV-positive status do not need to be retested. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment.

Table 6 “List of study procedures for Cycle 1 (including screening)” has been adapted to include this HIV test.

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Appendix A TNM classification for melanoma

The TNM staging system characterizes melanoma with respect to several factors including tumor size, thickness and ulceration, extent of lymphatic invasion, and the presence of distant metastasis.

The current version of the staging system used has been published in the Sixth Edition of the AJCC Cancer Staging Manual [American Joint Committee on Cancer, 2002].

The definition of TNM is the following [American Joint Committee on Cancer, 2002]:

T – Primary tumor

TX – Primary tumor cannot be assessed (e.g., shave biopsy or regressed melanoma).

T0 – No evidence of primary tumor

Tis – Melanoma *in situ*

T1 – Melanoma of ≤ 1.0 mm in thickness with or without ulceration

T1a – Melanoma of ≤ 1.0 mm in thickness and level II or III, no ulceration

T1b – Melanoma of ≤ 1.0 mm in thickness and level IV or V or with ulceration

T2 – Melanoma 1.01-2.0 mm in thickness with or without ulceration

T2a – Melanoma 1.01-2.0 mm in thickness, no ulceration

T2b – Melanoma 1.01-2.0 mm in thickness, with ulceration

T3 – Melanoma 2.01-4.0 mm in thickness with or without ulceration

T3a – Melanoma 2.01-4.0 mm in thickness, no ulceration

T3b – Melanoma 2.01-4.0 mm in thickness, with ulceration

T4 – Melanoma greater than 4.0 mm in thickness with or without ulceration

T4a – Melanoma > 4.0 mm in thickness, no ulceration

T3b – Melanoma > 4.0 mm in thickness, with ulceration

N – Regional lymph nodes

NX – Regional lymph nodes cannot be assessed

N0 – No regional lymph node metastasis

N1 – Metastasis in one lymph node

N1a – Clinically occult (microscopic) metastasis

N1b – Clinically apparent (macroscopic) metastasis

N2 – Metastasis in two to three regional nodes or intra-lymphatic regional metastasis without nodal metastases

N2a – Clinically occult (microscopic) metastasis

N2b – Clinically apparent (macroscopic) metastasis

N2c – Satellite or in-transit metastasis *without* nodal metastasis

N3 – Metastasis in four or more regional lymph nodes, or mated metastatic nodes, or in-transit metastasis or satellite(s) *with* metastasis in regional node(s)

M – Distant metastasis

MX – Distant metastasis cannot be assessed

M0 – No distant metastasis

M1 – Distant metastasis

M1a – Metastasis to skin, subcutaneous tissue or distant lymph node

M1b – Metastasis to lung

M1c – Metastasis to all other visceral sites or distant metastasis at any site associated with an elevated serum lactate dehydrogenase (LDH)

AJCC pathological stage groupings

Stage 0	Tis, N0, M0
Stage IA	T1a, N0, M0
Stage IB	T1b, N0, M0 T2a, N0, M0
Stage IIA	T2b, N0, M0 T3a, N0, M0
Stage IIB	T3b, N0, M0 T4a, N0, M0
Stage IIC	T4b, N0, M0
Stage IIIA	T1-4a, N1a, M0 T1-4a, N2a, M0
Stage IIIB	T1-4b, N1a, M0 T1-4b, N2a, M0 T1-4a, N1b, M0 T1-4a, N2b, M0 T1-4a/b, N2c, M0
Stage IIIC	T1-4b, N1b, M0 T1-4b, N2b, M0 Any T, N3, M0
Stage IV	Any T, Any N, M1

Appendix B Common Terminology Criteria for Adverse Events, Version 4.0

The severity of adverse events will be assessed by reference to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 of May 28, 2009. A copy can be downloaded from the internet website:

http://evs.nci.nih.gov/ftp1/CTCAE

Appendix C Eastern Cooperative Oncology Group (ECOG) performance status

0 Fully active, able to carry on all pre-disease performance without restriction.

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.

2 Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.

3 Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.

4 Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.

5 Dead.

Appendix D Amendments and administrative changes to the protocol

GlaxoSmithKline Biologicals	
Clinical Research & Development	
Protocol Amendment 1	
eTrack study number and abbreviated title	112406 (NYESO1-AS15-MEL-001 (MET))
EudraCT number	2010-020663-20
Title	Study of GSK2241658A Antigen-Specific Cancer Immunotherapeutic in patients with unresectable and progressive metastatic cutaneous melanoma
Detailed title:	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma
Amendment number:	Amendment 1
Amendment date:	18 April 2011
Co-ordinating author:	PPD (Scientific writer)

The background and rationale for the changes made to the protocol as a consequence of Amendment 1 are described below:

- Reduce the interval between Cycle 1 and Cycle 2 by one week with consequent changes of dates of ASCI administrations to align with other ASCI studies with the same treatment schedule (Synopsis, Sections 3.3 and 6.4)
- Ensure that tumor biopsies and imaging done as local standard practice or for another research study are not to be repeated if timelines are respected (Sections 4.2, 6.4, 6.5.2.1, 6.5.2.6)
- Specify quantitative limits for hematological variables (Section 4.2)
- Allow higher bilirubin values for patients with Gilbert's syndrome (Section 4.2)
- Update section on study product storage according to new protocol exemption form (Section 5.2)
- Recording of second-line treatment after disease progression and assessment of tumor response to this (Sections 5.5.2, 6.6.5)
- Specifying that tumor tissue samples from biopsies or resection of additional lesions during the ASCI administration period may be used to perform tests of NY-ESO-1 antigen expression and gene profile plus for optional translational research for patients having given specific informed consent to this (Sections 5.6.1 and 6.5.3.6)
- Clarification that pregnancy tests may be done following standard clinical practice using blood or urine test (Sections 6.4, 6.5.2.2.8, 6.5.3, 6.7.2)
- Clarification that alternation of ASCI administration side is preferable but not mandatory (Section 5.3)

- Reporting of auto-immune diseases has been replaced by reporting of potential immune-mediated diseases (pIMDs) and the list of autoimmune disease has been replaced by a list of pIMDs. It is specified that all pIMDs will be recorded in the eCRF/reported within 24 hours using the SAE screen, regardless of whether the pIMDs are AEs or SAEs. In addition, SAEs related to the study treatment are introduced as a separate category of adverse events, to be recorded within a specific time frame. Instructions for recording and reporting adverse events, pIMDs and pregnancies have been reshuffled and grouped for clarity. Referrals to autoimmune diseases have been replaced by pIMD throughout
- Some inconsistencies in the use of US English spelling have been corrected throughout the document

In the following sections, amended text has been included in *bold italics,* while deleted text is marked by ~~strikethrough~~:

Front page, Protocol amendment 1 Sponsor Signatory Approval and Protocol amendment 1 Investigator agreement:

IND number: 14313

Eudract Number: 2010-020663-20

PPD ~~, PhD (*Project Manager, Science Writing, Clinical Operations*~~

PPD ***, MSc, Scientific writer***

PPD ***, MSc (Global Study Manager, Clinical Operations, Cancer Immunotherapeutics)***

PPD ***, MD, Senior Manager, Clinical Safety and Pharmacovigilance***

PPD ***(Clinical Data Management, Cancer Immunotherapeutics)***

PPD ~~BSc (*Specialist, Clinical Data Management, Cancer Immunotherapeutics)*~~

Synopsis, Study design

Cycle 2: 6 ASCI administrations given at 3-week intervals
(Weeks 1***4***~~5~~, 1~~7~~8, 2***0***~~1~~, 2***3***4, 2***6***~~7~~, ***2***9~~30~~)

Cycle 3: 4 ASCI administrations given at 6-week intervals
(Weeks 3***3***4, ***3***9~~40~~, 4***5***~~6~~, 5***1***~~2~~)

List of abbreviations

pIMD ***potential immune mediate disease***

Section 3.1 General outline of study design

This will be an open-label, single-group, multicent~~re~~***er*** Phase I study, planned to be conducted at approximately 20 centers.

~~The Sponsor will in turn inform all the investigators within 24 hours by fax or email. The investigators must acknowledge receipt of this information.~~

Section 3.2 Study treatment schedule

Cycle 2:	6 ASCI administrations, each given at 3-week intervals (Weeks 1***4***~~5~~, 1***7***~~8~~, 2***0***~~1~~, 2***3***4, 2***6***~~7~~, ***29***~~30~~)
Cycle 3:	4 ASCI administrations, each given at 6-week intervals (Weeks 3***3***~~4~~, ***39***~~40~~, 4***5***~~6~~, 5***1***~~2~~)

Section 3.3 Duration of study participation

Cycle no.		***Cycle 2***							
Interval between ASCI administrations (admin)		**3 weeks**							
		Continue treatment if CR or PR or SD or SPD at each TE							
Visit no.	Screening	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Weeks after first ASCI injection	w-***6*** to w-1	1~~5~~***4***	1~~8~~***7***	2~~1~~***0***	2~~3~~***2***	24***3***	2~~7~~***6***	30***29***	3~~2~~***1***
recNY-ESO-1 + AS15 admin no.		**7**	**8**	**9**		**10**	**11**	**12**	

Cycle no.	***Cycle 3***				
Interval between ASCI administrations (admin)	**6 weeks**				
	Visit				
Visit no.	*16*	*17*	*18*	*19*	*20*
Time after first ASCI admin	3~~4~~***3*** w	40***39*** w	4~~6~~***5***w	5~~2~~***1***w	54w
recNY-ESO-1 + AS15 admin no.	**13**	**14**	**15**	**16**	

Section 4.1.1 Overview of the recruitment plan

- Recruitment is expected to start in ~~the October~~ ***February*** 201***1***~~0~~ and is expected to be completed within approximately 1 year, i.e., by the end of the ~~3~~rd ***1st*** quarter of 201***2***~~1~~. If this target cannot be met, the recruitment period may be extended as required.

Section 4.2 Inclusion criteria

Note: Procedures performed before obtaining the patient's informed consent as part of standard institution practices or in the context of another research study (see Section6.4) are accepted as study procedures provided the time intervals stipulated in the protocol are observed.

The patient has normal organ functions as shown by all of the following:

- Hemoglobin ≥ ~~Lower Limit of Normal.~~ ***12 g/dL***
- Absolute leukocytes count ≥ ~~Lower Limit of Normal.~~ ***3.0 x 10^9/L***
- Absolute lymphocytes count ≥ ~~Lower Limit of Normal.~~ ***1.0 x 10^9/L***
- Platelets ≥ ~~Lower Limit of Normal.~~ ***100 x 10^9/L***
- Serum creatinine ≤ Upper ~~Normal~~ Limit ***of Normal (ULN)***.
- Serum total bilirubin ≤ 1.5 x ~~Upper Normal Limit~~ ***ULN (except for patients with Gilbert's syndrome for whom the limit is 2 x ULN).***
- Lactate dehydrogenase (LDH) ≤ ~~Upper Normal Limit.~~ ***ULN***
- Aspartate aminotransferase (ASAT) ≤ 2 x ~~Upper Normal Limit~~. ***ULN***
- Alanine aminotransferase (ALAT) ≤ 2 × ~~Upper Normal Limit~~. ***ULN***

Section 4.3 Exclusion criteria

The patient has at any time received systemic chemotherapy, biochemotherapy, ***small molecules*** or anti-CTLA-4 monoclonal antibody for metastatic disease.

~~12. The patient has a family history of congenital or hereditary immunodeficiency.~~

Section 5.2 Storage and handling of the study product

All ASCI ***products*** to be administered to the patients must be stored in a safe and locked place with no access by unauthorized personnel.

The ASCI ***product must*** ~~will~~ be stored at the defined temperature range (i.e., 2 to 8°C/36°F to 46°F). Please refer to the Study Procedures Manual (SPM) for more details on storage of the ASCI. The storage temperature of the ASCI will be monitored daily with validated temperature monitoring device(s) ***(at the minimum calibrated)*** and will be recorded as specified in the SPM.

The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact.

Any temperature deviation~~, i.e. temperature~~ outside the ~~defined~~ range (***0***~~2~~-8°C/36-46°F), must be reported to the sponsor as soon as detected. Following an exposure to ***such*** a temperature deviation, ASCI will not be used until ~~written~~ approval has been given by the Sponsor. ***In case of temperature deviation between 0 and +2°C/32 and 36°F, the impacted study products can still be administered, but the site must take adequate actions to go back to the defined range +2 to +8°C/36 to 46°F and avoid re-occurrence of such a temperature deviation.***

Refer to the SPM for details and instructions on the ***temperature deviation process***, packaging and accountability of the ASCI.

Section 5.3.2 Administration

….The final preparation is to be injected immediately or, if not possible, no later than 4 hours after reconstitution and provided that the final preparation has been kept at a temperature between 4°C (39°F) and ***30***~~25~~°C (***86***~~77~~°F).

~~When reconstituted, the recNY-ESO-1 + AS15 ASCI can be kept between 4°C (39°F) and 25°C (77°F) for a maximum of 4 hours.~~

Table 2 Dosage and administration

Treatment	Dose	Administration	
		Timing	***Route and site***
recNY-ESO-1 + AS15 ASCI	1 dose corresponding to 300 μg of recNY-ESO-1 protein and 420 μg CpG, reconstituted in AS01B-4	Cycle 1: q2w x 6 Cycle 2: q3w x 6 Cycle 3: q6w x 4 Cycle 4: q3m x 4 then q6m x 4	i.m. Deltoid or lateral region of the thigh, ***preferably*** with alternation on right or left side at each succeeding injection

Section 5.5.2 Criteria for permanent stopping of ASCI administration

After disease progression, the choice of subsequent treatment will be at the discretion of the treating physician; the patient will be taken off study treatment and the investigator will document ~~at least the first-line treatment administered after the study.~~ ***any further anti-cancer treatment administered and assess the patient's best clinical response to second-line treatment.***

5.6.1 Permitted medication

~~Treatment of any melanoma progression~~ ***The following palliative melanoma treatments*** (***of*** tumor sites or symptoms) ***are*** allowed in addition to the study treatment ~~are~~

- Surgery.

Note: If surgery is performed, sample(s) of the resected tumor lesion(s) will be sent to GSK Biologicals or a laboratory contracted for this by GSK Biologicals (see Sections 7.1 and 7.2.2). For patients who have given their specific informed consent to the optional translational research, such tumor tissue(s) may also be used to conduct this specific testing (see Sections 7.3, 7.6, 7.7, and 7.8).

Section 6.1.1 Patient identification

Patient numbers will be assigned sequentially to patients consenting to participate in the study, and according to the range of patient numbers allocated to each study cent***er***~~re~~.

Section 6.4 Outline of study procedures

Table 6. List of study procedures for Cycle 1

Visit no.	Screening a	1	2	3	4	5	6	7
Weeks after first ASCI administration	-	0	2	4	6	8	10	12
NY-ESO-1 ASCI admin no.	-	1	2	3	4	5	6	
Safety assessments								
(Serious) Adverse Events recorded d	●	●	●	●	●	●	●	●
Potential immune-mediated diseases (pIMDs) recorded		●	●	●	●	●	●	●

Table 7. List of study procedures for Cycle 2

Visit no.	8	9	10	11	12	13	14	15
Weeks after first ASCI administration	1~~45~~	1~~78~~	2~~01~~	2~~23~~	2~~34~~	2~~67~~	2~~930~~	3~~12~~
NY-ESO-1 ASCI admin no.	7	8	9		10	11	12	

Table 8: List of study procedures for Cycle 3

Visit no.	16	17	18	19	20
Weeks after first ASCI administration	3~~34~~	~~39~~40	4~~56~~	5~~12~~	54
NY-ESO-1 ASCI admin no.	13	14	15	16	

Tables 6 - 10 List of study procedures

_Safety assessments								
~~Autoimmune diseases~~ *pIMDs* recorded		●	●	●	●	●	●	●
Laboratory assessments								

Footnotes to Table 6 (only footnotes impacted are retained here)

a. Screening visit/period is to take place a maximum of 6 weeks before the first ASCI administration. However:

1. Urine and blood chemistry, hematological, coagulation and autoimmunity-tests within 3 weeks before the first ASCI administration;
    2. Negative ~~urine~~ pregnancy test must be obtained within 1 week before the first ASCI administration for women of child-bearing potential;
    3. Tumor biopsy can be obtained within 12 weeks before the first ASCI administration; in the rare case the analysis of the sample would give an inconclusive result, the patient may be asked to allow a new sample to be collected or to allow the use of an already preserved sample to repeat the test. ***A tumor biopsy performed in the context of institution standard practice or another research study may be used for the NY-ESO-1 testing after obtaining the Sponsor's agreement, provided this biopsy was preserved in RNAlater and was taken no more than 12 weeks before the first administration of the study treatment.***
    4. ***Tumor i***maging no more than 6 weeks before first ASCI administration. ***Imaging performed as part of institution standard clinical practice or in relation to another research study does not need to be***

repeated, provided this imaging was performed no more than 6 weeks before the first administration of the study treatment.

Section 6.5.2.1 NY-ESO-1 expression screening of tumor lesion(s)

The tumor biopsy could have been done previously in the context of local routine practices ***or as part of another research study***. This tumor material may be used for the NY-ESO-1 expression analysis if and only if:

- The patient signs the study ICF before the tumor sample is sent to GSK Biologicals for analysis, and;
- The first ASCI administration is to take place no more than 12 weeks after this tumor biopsy.
- ***The Sponsor's permission to use this tissue has been obtained in advance.***
- ***The tumor tissue has been preserved in RNAlater immediately after the surgery and*** the amount of tissue available must comply with the protocol requirements (see ***the*** SPM).

Section 6.5.2.6 Tumor imaging and assessment

Note: Tumor imaging performed as part of standard clinical practice or in relation to another research study does not need to be repeated, provided this imaging was done no more than 6 weeks before the first administration of the study treatment.

Section 6.5.2.7 Urine and blood collection

- Collect a urine sample for urine chemistry tests including microalbuminuria. ***A validated quantitative test can be used according to local practice; for each individual patient the same test method must be used for all assessments.***

Section 6.5.2.8 Pregnancy tests

Female patients of childbearing potential are to have a pregnancy test ~~(urine or blood test according to local practice)~~ at screening and visits 1, 2, 4 and 6 during Cycle 1.

Section 6.5.3 Procedures during ASCI administration phase

Note that some of the procedures to be performed during the ASCI administration period (such as the physical examination, tumor imaging and assessment, taking urine samples, taking blood samples for hematological tests, blood chemistry tests, autoimmunity tests tests and coagulation tests, and ~~urine~~ pregnancy tests) are also performed during screening and are described in Section 6.5.2.

Section 6.5.3.6 Additional tumor biopsy or tumor resection

If surgery is performed during the ASCI administration phase (see Section 5.6.1), sample(s) of the resected tumor lesion(s) will be sent to GSK Biologicals or a laboratory contracted for this by GSK Biologicals (see Sections 7.1 and 7.2.2). For patients who have given their specific informed consent to the optional translational research, such tumor tissue(s) may also be used to conduct this specific testing (see Sections7.3, 7.6, 7.7, and 7.8).

Section 6.5.3.9 Recording of non-serious AEs, SAEs, ~~autoimmune diseases~~ ***potential immune-mediated diseases (pIMDs)*** and pregnancy

Refer to Section 9.3 for procedures for the investigator to record AEs and SAEs and to Section 9.4 for guidelines on how to report these AEs/SAEs to GSK Biologicals. Refer to Section 9.4.5 for instructions on reporting of ~~autoimmune diseases~~ ***pIMDs***.

Section 6.5.4.1 Patients who received all the scheduled doses and

Section 6.5.4.2 Patients withdrawn before the end of the scheduled doses

At the visit, the following procedures described above will be performed: physical examination; recording any AEs and SAEs, ~~autoimmune diseases~~ ***pIMDs***, and concomitant medications; taking a urine sample for urine chemistry tests; taking a blood sample for hematological tests, blood chemistry tests, autoimmunity tests, coagulation tests, serum sampling for humoral immunity tests and PBMC collection for cellular immunity tests.

For patients who have withdrawn because of progression of the disease, any resected tumor can be sent to GSK for analysis of ***NY-ESO-1 expression and*** gene profile upon signature of the adequate ICF by the patient (refer to Section 12.2); ***for patients who have given their specific consent to this, such tumor tissue(s) may also be used for optional translational research.***

Section 6.5.5 Procedures during follow-up phase

- Patients who were withdrawn from study treatment because of disease progression or who progressed during the post-treatment study period will not be asked to return for complete study follow-up visits. These patients will be ~~contacted every 6 months after the first ASCI injection for survival assessment until 5 years after the first study treatment administration.~~ ***followed for survival by means of bi-annual contacts (e.g., by phone). If a second-line anti-cancer treatment is administered, the patient's best clinical response to this will be assessed, e.g., during the patient's standard visits to the institution.***

Section 6.7.2 Hematology, serum chemistry, urine tests

Sample type	System	Component	Scale	Timing
PREGNANCY TESTING (MANDATORY)				
Blood (5 ml) or urine according to local practice	Serum or urine	~~Choriogonadotropin (urine HCG pregnancy test)~~ ***According to local practice***	Ordinal	During screening Cycle 1: Visits 1, 2, 4, and 6 Cycles 2 - 4: prior to each ASCI administration

Section 6.7.3 Molecular, immunological and translational research read-outs

***NY-ESO-1* SCREENING (MANDATORY)**			
Test	**Sample Type**	**Laboratory**	**Timing**
***NY-ESO-1* expression testing** (see also Section 6.7.3.1)	Fresh tissue sample (in *RNAlater*® or equivalent)	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). ***In case of additional tumor biopsy or tumor resection***
MANDATORY TRANSLATIONAL RESEARCH (SEE SECTION 7.2.1)			
Test	**Sample Type**	**Laboratory**	**Timing**
Gene profiling of the tumor	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d]	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection. ~~(disease progression)~~
OPTIONAL TRANSLATIONAL RESEARCH (EXCEPT SECTION 7.2.1)			
Test	**Sample Type**	**Laboratory**	**Timing**
Expression analysis of NY-ESO-1 and other tumor antigens (e.g., MAGE-A3, PRAME, LAGE-1, MAGE-C2 and WT1)	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d]	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection. ~~(disease progression)~~
Antigen spreading	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy). In case of additional tumor biopsy or tumor resection. ~~(disease progression)~~ May be performed on any of the blood samples taken for the humoral and cellular immunity tests
Proteomic profiling	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy) or on any of the blood samples taken for the humoral and cellular immunity tests
Tumor infiltrating lymphocytes (including T regulatory cells and other immune cellular entities)	Either fresh tissue sample (in *RNAlater*® or equivalent) or FFPE tissue (if available) [d]		During screening (initial biopsy). In case of additional tumor biopsy or tumor resection. ~~(disease progression)~~

7.2.2 Gene profiling and NY-ESO-1 expression testing on additional lesions

These tests will be performed ~~**I**~~***i***n case the investigator ~~will~~ ***plans to*** perform or has performed an additional tumor biopsy or a tumor resection ***during the study (e.g. palliative surgery, see Section 5.6.1) or*** because of disease progression. ***If the procedure occurs in the context of disease progression, the*** patients will be proposed to sign a separate ICF to allow a sample of this lesion to be sent to GSK Biologicals or a validated laboratory designated by GSK Biologicals for the optional research on gene profiling.

7.6 Analysis of tumor immune filtration

This analysis will be done using either fresh tumor tissue or FFPE tumor samples if available at screening and later biopsies collected ~~upon~~ ***during the study and at*** disease progression.

7.7 DNA promoter methylation analysis

Tumor cell DNA can be detected from the blood of melanoma patients even in early disease [Kounalakis, 2005]. This DNA can for instance originate from the primary tumor, where some cells are ly~~z~~s~~e~~d releasing DNA material in the blood or from circulating tumor cells.

A fraction of the stored serum and blood collected over time for the immunological analyses will be used for the methylation analysis. DNA methylation will also be assessed either on fresh tumor tissue or FFPE tumor sections used for NY-ESO-1 detection and ~~biopsy of any new lesions.~~ ***additional biopsies taken during the study and at disease progression.***

7.8 Pharmacogenetics analyses

- For the tumor genetic variability: this analysis might be performed on the tumor sample collected for screening, on tumor ***tissue*** samples from ***additional resected*** ~~of progressive~~ lesions or on tumor invaded lymph node when material is available and of appropriate quality. Of note, this investigation of the tumoral cells will not require additional material

Section 9.1.2 Definition of a serious adverse event

c. Requires hospitali~~z~~s~~e~~ation or prolongation of existing hospitali~~z~~s~~e~~ation.

NB: In general, hospitali~~z~~s~~e~~ation signifies that the patient has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitali~~z~~s~~e~~ation are also considered AEs. If a complication prolongs hospitali~~z~~s~~e~~ation or fulfills any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitali~~z~~s~~e~~ation' occurred or was necessary, the AE should be considered serious.

g. In addition, in this study, Grade 3 or higher ~~autoimmune disorders~~ ***pIMDs*** will be considered as medically significant and will therefore be notified as SAE.

h. Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

Section 9.1.3 ~~Autoimmune diseases~~ ***Potential immune mediated diseases***

Potential immune mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in the table below.

Neuroinflammatory disorders	*Musculoskeletal disorders*	*Skin disorders*
Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy), and neuritis (e.g. optic neuritis) *Multiple sclerosis (including variants)* *Transverse myelitis* *Guillain-Barré syndrome, (including Miller Fisher syndrome and other variants)* *Other demyelinating diseases (including acute disseminated encephalomyelitis)* *Myasthenia gravis (including Lambert-Eaton myasthenic syndrome)* *Non-infectious encephalitis/ encephalomyelitis* *Neuritis (including peripheral neuropathies)*	*Systemic lupus erythematosus* *Scleroderma (including, CREST syndrome and morphoea)* *Systemic sclerosis* *Dermatomyositis* *Polymyositis* *Antisynthetase syndrome* *Rheumatoid arthritis,* *Juvenile chronic arthritis, (including Still's disease)* *Polymyalgia rheumatica* *Reactive arthritis* *Psoriatic arthropathy* *Ankylosing spondylitis (including undifferentiated spondyloarthritides)* *Relapsing polychondritis* *Mixed connective tissue disorder*	*Psoriasis* *Vitiligo* *Raynaud's phenomenon* *Erythema nodosum* *Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)* *Cutaneous lupus erythematosus* *Alopecia areata* *Lichen planus* *Sweet's syndrome*
Liver disorders	*Gastrointestinal disorders*	*Metabolic diseases*
Autoimmune hepatitis *Primary biliary cirrhosis* *Primary sclerosing cholangitis* *Autoimmune cholangitis.*	*Crohn's disease* *Ulcerative colitis* *Ulcerative proctitis* *Celiac disease*	*Autoimmune thyroiditis (including Hashimoto thyroiditis)* *Grave's or Basedow's disease* *Diabetes mellitus type I* *Addison's disease*

Vasculitides	*Others*
• *Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.* • *Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome, thromboangiitis obliterans (Buerger's disease), necrotizing vasculitis, allergic granulomatous angiitis, Henoch-Schonlein purpura, anti-neutrophil cytoplasmic antibody positive vasculitis, Behcet's syndrome, leukocytoclastic vasculitis.* • *Vasculitides secondary to other immune mediated diseases such as lupus vasculitis and rheumatoid vasculitis.*	• *Autoimmune hemolytic anemia* • *Autoimmune thrombocytopenias* • *Antiphospholipid syndrome* • *Pernicious anemia* • *Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)* • *Uveitis* • *Autoimmune myocarditis/cardiomyopathy* • *Sarcoidosis* • *Stevens-johnson syndrome* • *Sjögren's syndrome* • *Idiopathic pulmonary fibrosis* • *Goodpasture syndrome*

However, the investigator will exercise his/her medical and scientific judgment in deciding whether other immune-mediated diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

When there is enough evidence to make any of the diagnoses listed in Table 13, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent one of these diagnoses, should be recorded and reported as AEs but not as a pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

In order to facilitate the documentation of pIMDs in the eCRF, a pIMD standard questionnaire and a list of preferred terms (PTs) and PT codes corresponding to these diagnoses will be available to the investigators at the study start.

Section 9.1.4 Clinical laboratory parameters……

Abnormal laboratory findings (e.g. clinical chemistry, h~~ae~~matology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will

Section 9.3.1 Time period for detecting and recording of adverse events.....

All AEs (except ~~autoimmune~~ AEs ***categorized as pIMDs*** - see Section 9.1.3) starting from first dose to 30 days after the last dose of the ASCI must be recorded into the Adverse Event screen in the patient's eCRF, irrespective of intensity or whether or not they are considered ASCI administration-related.

The standard time period for collecting and recording SAEs (except ~~autoimmune~~ ***SAEs categorized as pIMDs*** - see Section 9.1.3) will begin at the first receipt of ASCI and will end 30 days following administration of the last dose of ASCI for each patient. See Section 9.4 for instructions on reporting and recording SAEs.

The standard time period for collecting and recording severe toxicity events, ~~autoimmune diseases~~ ***pIMDs*** and pregnancy will begin at the first receipt of the product and last until the end of the study (Table 14).

Table 14 Reporting periods for AEs, ~~autoimmune diseases~~ ***pIMDs***, …….

Study activity	Screening	Treatment period		Concluding visit	Follow-up
	From ICF signature to Visit 1	Visit 1	Visit X (X=2 to 13)	Visit 14 or 30 days after last ASCI administration	1 year after concluding visit
Reporting of severe toxicity events					
Reporting of ***pIMDs*** and pregnancy					
Reporting of SAEs related to the study treatment					

Section 9.3.2.2.1 Assessment of intensity

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets the definition in Section 9.1.2.

Section 9.4.1 Prompt reporting of serious adverse events and other events to GSK Biologicals

Table 15

Type of Event	Initial Reports		Follow-up Information on a Previous Report	
	Time Frame	**Documents**	**Time Frame**	**Documents**
All SAEs	24 hours*	SAE screen	24 hours*	SAE screen
Severe toxicities	***24 hours****	***eCRF***	***24 hours****	***eCRF***
pIMDs	***24 hours***	***SAE screen***	***24 hours***	***SAE screen***
Pregnancy	24 hours*	Pregnancy Report Form	24 hours*	Pregnancy Report Form

* Time frame allowed after receipt or awareness of the information.

Back-up Study Contact for Reporting SAEs *and severe toxicities*
GSK Biologicals Clinical Safety & Pharmacovigilance Fax: +PPD or +PPD **24/24 hour and 7/7 day availability**

9.4.3.1 Back-up system in case the electronic SAE reporting system does not work

~~In rare circumstances, if the electronic system for reporting SAEs (including deaths determined by the investigator to be related to the ASCI administration) does not work and in the absence of facsimile equipment, notification by email is acceptable. Initial notification via email does not replace the need for the investigator to complete and submit SAE screens in the eCRF (or complete and sign the SAE Report Form if back-up system need to be used).~~ ~~The email address for reporting purposes is:~~PPD PPD

9.4.3.2 Back-up system in case the electronic system for reporting of severe toxicities does not work

If the electronic system for severe toxicity reporting has been down for 24 hours, the investigator or his/her delegate should fax a severe toxicity form directly to the GSK Biologicals Central Safety Department (please refer to Section 9.4.1) within 24 hours. The maximum timeline for reporting severe toxicities is therefore 48 hours.

As soon as the electronic reporting system is working again, the investigator or delegate must update the severe toxicity screen in the eCRF within 24 hours.

The final valid information for regulatory reporting will be the information reported through the electronic system.

9.4.4 Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a patient is pregnant, the investigator (or designate) must complete a Pregnancy Report Form and fax it to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) WITHIN 24 HOURS.

The Pregnancy Report Form will always be completed as thoroughly as possible with all available details and then dated and signed by the investigator (or designate). Even if the investigator does not have all information regarding the pregnancy, the form should still be completed and forwarded to GSK within 24 hours. Once additional relevant information is received, the form will be updated and forwarded to GSK WITHIN 24 HOURS.

In absence/dysfunction of facsimile equipment, the Study Contact for Reporting SAEs should be notified by telephone within 24 hours. As soon as the facsimile equipment is working again, the investigator (or designate) must fax the Pregnancy Report Form to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) within 24 hours.

9.4.5 Reporting of pIMDs to GSK Biologicals

Once onset of a new pIMD or exacerbation of a pre-existing pIMD is diagnosed (serious or non-serious) in a study patient, the investigator (or designate) must complete the information in the SAE screens of the eCRF WITHIN 24 HOURS after he/she becomes aware of the diagnosis. A field on the SAE screen allows to specify that the event is a pIMD and whether it is serious or non serious. The SAE screens will always be completed as thoroughly as possible with all available details of the event, in accordance with the pIMD standard questionnaire provided. Even if the investigator does not have all information regarding a pIMD, the SAE screens should still be completed within 24 hours. Once additional relevant information is received, the SAE screens in the eCRF should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

Refer to Section 9.4.3.1 for back-up system and updating of SAE information after freezing of the patient's eCRF.

Section 9.4.6 Follow-up of adverse events and serious adverse events

All AEs and ~~autoimmune diseases~~ ***pIMDs*** documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last ASCI administration.

All severe toxicities and ~~autoimmune diseases~~ ***pIMDs*** documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the end of the study.

Investigators will follow-up patients:

- With SAEs or patients withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabili~~z~~sed, disappeared, or until the event is otherwise explained, or the patient is lost to follow-up.
- Or, in the case of other non-serious AEs and ~~autoimmune diseases~~ ***pIMDs*** until they complete the study or they are lost to follow-up.

If a patient dies during participation in the study or during a recogni~~z~~sed follow-up period, GSK Biologicals will be provided with a copy of any available post-mortem findings, including histopathology.

Section 12.1 Remote data entry instructions

If the authori~~z~~sed individuals change during the study, the investigator is to inform GSK Biologicals' representative of the specific change(s). The SSSS needs to be updated accordingly in order to reflect the current situation at all times.

12.8.3 Publication

For multicent~~reer~~ studies, , the first publication or disclosure of study results shall be a complete, joint multicent~~reer~~ publication or disclosure coordinated by GSK. Thereafter, any secondary publications will reference the original publication(s).

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Administrative change 1

eTrack study number(s) and abbreviated title(s)	112406 (NYESO1-AS15-MEL-001 (MET))
EudraCT number	2010-020663-20
Title	Study of GSK2241658A Antigen-Specific Cancer Immunotherapeutic in patients with unresectable and progressive metastatic cutaneous melanoma
Detailed Title	**An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma**
Administrative change date:	Administrative change 1 Final: 17 January 2013
Co-ordinating author:	PPD Ph.D, (*Scientific Writer, Cancer Immunotherapeutics*)

In the following sections, additions are marked by ***bold/italic***, while deleted text is marked by ~~strikethrough~~:

Cover page

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Sponsor signatory

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Signature

Protocol Administrative Change 1 Investigator Agreement

Investigator name _____

Signature _____

Date _____

PPD

(LKP) name: _____

Signature _____

Date _____

Section 13

~~Not Applicable~~

13.1 French administrative considerations

This Section includes all the requirements of the French law (n° 2004-806 of 09 August 2004), and identifies, item by item, the mandatory modifications or additional information to the study protocol.

- ***Concerning the "Study Population"***

  In line with the local regulatory requirements, the following text in section "OTHER STUDY ELIGIBILITY CRITERIA CONSIDERATIONS" is added:

  A patient will be eligible for inclusion in this study if he /she is either affiliated to or beneficiary of a social security category.

  It is the investigator's responsibility to ensure and to document (in source document - patient notes) that the patient is either affiliated to or beneficiary of a social security category.

- ***Concerning the "Data Evaluation: Criteria for Evaluation of Objectives"***

  Section on the "ESTIMATED SAMPLE SIZE"

  The following text concerning the "sample size assumption" is added:

  The expected number of patients to be recruited in France is declared to the French Regulatory Authority.

- ***Concerning the "Conduct of the Study"***
  Section "REGULATORY AND ETHICAL CONSIDERATIONS, INCLUDING THE INFORMED CONSENT PROCESS"

  The following text concerning "the process for informing the patient or his/her legally authorized representative" is added:

  - ***French Patient ICF is a document in triplicate which summarizes the main features of the study and allows collection of the patient's written consent. It also contains a reference to the authorization of ANSM and the approval from the French Ethics Committee and the maintenance of confidentiality of the returned consent form by GSK France.***

  In addition, the following text concerning "the process for obtaining the patient's informed consent" is added:

  - ***When biomedical research is carried out on a minor / on an adult in the care of a "tutelle" guardian, consent is given by their legal representative and, if the committee mentioned in article L. 1123-1 considers that the research in question, because of the gravity of the restraints or the specificity of the medical acts involved, entails a serious risk of affecting their private life or the integrity of their body, by the family council if it has been instated, or by the judge of "tutelle" guardians.***
  - ***When biomedical research is carried out on an adult in the care of a "curatelle" guardian, consent is given by the patient assisted by his guardian. However, if the adult in the care of a "curatelle" guardian is invited to participate in research which the committee mentioned in article L. 1123-1 considers, because of the gravity of the restraints or the specificity of the medical acts involved, to entail a serious risk of affecting their private life or the integrity of their body, the matter is submitted to the judge of guardians who decides whether the adult is capable of giving his consent. In the case of incapacity, the judge will decide whether or not to authorize the biomedical research.***
  - ***When biomedical research, which complies with the conditions laid down in article L. 1121-8, is considered for an adult incapable of expressing his consent and not under a legal protection order, consent is given by a person of confidence as defined in article L. 1111-6 and, failing this, by a person who maintains close and stable links with the patient. However, if the committee mentioned in article L. 1123-1 considers that the research in question,***

because of the gravity of the restraints or the specificity of the medical acts involved, entails a serious risk of affecting their private life or the integrity of their body, consent is given by the judge of guardians.

- ***Finally, concerning "the management of the Patient ICFs" the following text is added:***

  The first copy of the Patient ICF is kept by the investigator. The second copy is kept by the Director of the Medical Department of GlaxoSmithKline France and the last copy is given to the patient or his/her legally authorized representative.

  The second copy of all the consent forms will be collected by the investigator at the end of the trial under the Clinical Research Assistant's (CRA's) control, and placed in a sealed envelope bearing only:

  - ***The study number,***
  - ***The identification of the Centre: name of the principal investigator and number of centre),***
  - ***The number of informed consents,***
  - ***The date and,***
  - ***The principal investigator's signature.***

  Then, the CRA hands the sealed envelope over to the Director of the Medical Department, for confidential recording, under his responsibility.

- ***In section concerning the " DEMOGRAPHIC DATA " the following text is added:***

  In accordance with the data-processing and freedom French law dated on 6th of January 1978 modified on the 6th of August 2004 - article 8, the ethnic origin can only be collected if the collection of this data is justified within the framework of this study.

- ***In section concerning the " TESTING OF BIOLOGICAL SAMPLES " the following text is added:***

  In accordance with Article L1211-2 of the French Public Health Code, a biological sample without identified purpose at the time of the sample and patient's preliminary information is not authorized.

- ***Concerning "Administrative matters"***

  The following text concerning "notification to the hospital director" is added (if applicable):

  In accordance with Article L1123-13 of the Public Health Code, the Hospital Director is informed of the commitment to the trial in his establishment. The Hospital Director is supplied with the protocol and any information needed for the financial disposition, the name of the investigator(s), the number of sites involved in his establishment and the estimated time schedule of the trial (R.1123-63).

The following text concerning "information to the hospital pharmacist" is added (if applicable):

In accordance with Article R.1123-64 of the Public Health Code, the Hospital Pharmacist is informed of the commitment to the trial in his establishment. The Pharmacist is supplied with a copy of the protocol (which allows him to dispense the drug(s) of the trial according to the trial methodology), all information concerning the product(s) of the trial (e.g. included in the Clinical Investigator's Brochure), the name of the investigator(s), the number of sites involved in his establishment and the estimated time schedule of the trial.

Finally, the following text concerning "data management" is added:

Within the framework of this clinical trial, data regarding the identity of the investigators and/or co-investigators and/or the pharmacist if applicable, involved in this clinical trial, and data regarding the patients recruited in this clinical trial (patient number, treatment number, patient status with respect to the clinical trial, dates of visit, medical data) will be collected and computerized in GSK Biologicals data bases by Laboratoire GlaxoSmithKline or on its behalf, for reasons of follow up, clinical trial management and using the results of said clinical trial. According to the Act no. 78-17 of 6th January 1978 further modified, each of these people aforesaid has a right of access, correction and opposition on their own data through Laboratoire GlaxoSmithKline (Clinical Operations Department).

GlaxoSmithKline Biologicals	
Clinical Research & Development	
Protocol Amendment 2	
eTrack study number and abbreviated title	112406 (NYESO1-AS15-MEL-001 (MET))
EudraCT number	2010-020663-20
Title	Study of GSK2241658A Antigen-Specific Cancer Immunotherapeutic in patients with unresectable and progressive metastatic cutaneous melanoma
Detailed title:	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma
Amendment number:	Amendment 2
Amendment date:	27 November 2013
Co-ordinating author:	PPD, PhD, *(Scientific writer, XPE Pharma for GSK Biologicals)*
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The background and rationale for the changes made to the protocol as a consequence of Amendment 2 are described below:

- Upon request from Paul-Ehrlich-Institut (Germany), testing for HIV has been made mandatory before study entry for all patients in German sites. All HIV-positive patients will be ineligible to enter the study. On account of this request, the following changes have been made in the protocol as part of this amendment.
  - A note has been added to exclusion criterion 10 in Section 4.3. This note is a specification for Germany only.
  - The HIV test has been included in the study procedures in Section 6.5.2.9, Section 6.7.2 and Table 6.
  - Section 13.2 German specific requirements has been created. This section contains a summary information regarding the HIV test.
- Administrative changes and minor modifications have been implemented.
  - The list of contributing authors has been updated.
  - Wording regarding the use of prednisone or equivalent has been clarified in Sections 4.3, 5.5.2 and 5.6.2.1.
  - Additional information regarding the tumor assessment has been added in section 6.5.2.6.
  - In Section 6.5.3.6, additional information regarding the need to label every new tumor biopsy/resection has been added. Information regarding the resected/biopsied tumor lesion(s) will be sent along with the lesion(s). This will include the nature of the lesion (skin, lymph node or other), if the lesion is new or not, if the lesion is part of the target lesions or not, if the lesion is inflammatory or not, if vitiligo is present around the lesion or not and the status of the lesion when resected/biopsied (progressive, stable or regressive).
  - The link to RECIST criteria information has been modified in Section 6.6.1. The previous link was not accessible on the internet anymore.
  - In section 11.2.3, the reference to “Section 6” has been changed by “Section 7”, as it is more convenient.
- The following notable errors have been corrected
  - Table 1 has been corrected to be aligned with Tables 6 to 9.
- Orthographic and grammatical typos have been corrected throughout the document.

In the following sections, amended text has been included in *bold italics,* while deleted text is marked by ~~strikethrough~~.

Cover page

PPD, MD, (***Vice-President***, Head of Early Clinical Development, Cancer Immunotherapeutics)

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List of abbreviations

DERMA	A**D**juvant Immunoth**E*R***apy with MAGE-A3 in melano***MA***

Table 1. Overview of ASCI administrations, tumor and immunological evaluations

Cycle no.		***Cycle 1***							***Cycle 2***							
Interval between ASCI administrations (admin.)		**2 weeks**							**3 weeks** *Continue treatment if CR or PR or SD or SPD at each TE*							
Visit no.	Screening	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Weeks after first ASCI admin (w)	~~w-3~~ ***w-6*** to w-1	0	2	4	6	8	10	12	14	17	20	22	23	26	29	31
recNY-ESO-1 + AS15 admin no.		**1**	**2**	**3**	**4**	**5**	**6**		**7**	**8**	**9**		**10**	**11**	**12**	
Tumor evaluation (TE)	**X**							**X**				**X**				**X**
Anti-NY-ESO-1 and anti-CpG7909 Ab response		**X**		**X**		**X**	***X***	***X***							**X**	
Anti-NY-ESO-1 T cell responses		**X**					***X***								**X**	

Cycle no.	***Cycle 3***					***Cycle 4***							
Interval between ASCI	**6 weeks**					**3 months**				**6 months**			**Conc Visit**
	Continue treatment if CR or PR or SD or SPD at each TE												
Visit no.	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*
Week (w), month (m) or year (y) after first ASCI admin.	33 ***w***	39 ***w***	45 ***w***	51 ***w***	54 ***w***	~~1y 3m~~ ***V19 +3m***	~~1y 6m~~ ***V21 +3m***	~~1y9m~~ ***V22 +3m***	~~2y~~ ***V23 + 3m***	~~2y 6m~~ ***V24 + 6m***	~~3y~~ ***V25 +6m***	~~3y 6m~~ ***V26 +6m***	~~4y~~ ***V27 +6m***	~~4y1m~~ ***V28 + 30 days***
recNY-ESO-1 + AS15 admin no.	**13**	**14**	**15**	**16**		**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	
Tumor evaluation (TE)					**X**		**X**		**X**	**X**	**X**	**X**		**X**
Anti-NY-ESO-1 and anti-CpG7909 Ab response				**X**			**X**		**X**	**X**	**X**	**X**		**X**
Anti-NY-ESO-1 T cell responses				**X**			**X**		**X**	**X**	**X**	**X**		**X**

Section 4.3. Exclusion criteria for enrolment

4. The patient requires concomitant treatment (more than 7 consecutive days) with systemic corticoseri~~o~~***oi***ds, or any other immunosuppressive agents.

Exception: The use of prednisone, or equivalent, at a dose ~~of~~ ≤ 0.125 mg/kg/day (absolute maximum 10 mg/day) ***for more than 7 consecutive days or at a dose > 0.125 mg/kg/day but for less than 7 consecutive days is allowed. The use of***~~,~~ inhaled corticosteroids or topical steroids is ***also*** permitted.

10. The patient is known to be positive for the Human Immunodeficiency Virus (HIV).

Specification for Germany: Please, refer to Section 13.2.

Section 5.5.2. Criteria for permanent stopping of ASCI administration

7. Appearance of any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection, or any medical condition requiring chronic treatment (more than 7 consecutive days) with systemic corticosteroids or any other immunosuppressive agents.

[Note: The use of prednisone, or equivalent, at a dose ~~of~~ ≤ 0.125 mg/kg/day (absolute maximum 10 mg/day) ***for more than 7 consecutive days or at a dose > 0.125 mg/kg/day but for less than 7 consecutive days is allowed. The use of***~~, or~~ inhaled corticosteroids or topical steroids is ***also*** permitted.]

Section 5.6.2.1. Prohibited medications or non-drug therapies

b. Administration of immunosuppressants or other immune-modifying drugs during the study period. The use of prednisone, or equivalent, at a dose ~~of~~ ≤ 0.125 mg/kg/day (absolute maximum 10 mg/day***) for more than 7 consecutive days or at a dose > 0.125 mg/kg/day but for less than 7 consecutive days is allowed. The use of***~~, maximum duration of treatment of one week) or~~ inhaled corticosteroids or topical steroids is ***also*** permitted

Table 6. List of study procedures for Cycle 1 (including screening)

Visit no.	Screening [a]	1	2	3	4	5	6	7
Weeks after first ASCI administration	-	0	2	4	6	8	10	12
NY-ESO-1 ASCI admin no.	-	1	2	3	4	5	6	
Laboratory assessments								
HIV status [i]	●							

8. HIV test within 3 weeks before the first ASCI administration, mandatory in Germany only (refer to Section 13.2).

i. HIV test is mandatory to determine the HIV status (positive - negative) of patients in Germany only. Patients with known HIV-positive status do not need to be re-tested. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment (refer to Section 13.2).

Section 6.5.2.6. Tumor imaging and assessment

Source documentation that is considered necessary by GSK Biologicals for efficacy or safety evaluation such as but not limited to pictures, CT-scans, histology, laboratory reports will be collected and upon request sent to GSK Biologicals for review, ensuring that patient confidentiality is maintained.

Section 6.5.2.9. HIV status (New section)

HIV test is mandatory in Germany only. Please, refer to Section 13.2.

Section 6.5.3.6. Additional tumor biopsy or tumor resection

In case the investigator performs an additional tumor biopsy or tumor resection during the ASCI administration phase ~~If surgery is performed during the ASCI administration phase~~ (see Section 5.6.1), sample(s) of the resected tumor lesion(s) will be sent to GSK Biologicals or a laboratory contracted for this by GSK Biologicals (see Sections 7.1 and 7.2.2). For patients who have given their specific informed consent to the optional translational research, such tumor tissue(s) may also be used to conduct ~~this~~ ***these*** specific ***tests*** ~~testing~~ (see Sections 7.3, 7.6, 7.7, and 7.8).

Information will be provided regarding the additional resected/biopsied lesion(s). This will include the nature of the lesion (skin, lymph node, other), if the lesion is new or not, if the lesion is part of the target lesions or not, if the lesion is inflammatory or not, if vitiligo is present around the lesion or not and the status of the lesion when resected/biopsied (progressive, stable or regressive).

Section 6.6.1. Response criteria for patients with all target lesions ≥ 20.0 mm

The following paragraphs contain a brief summary of the RECIST criteria [Therasse, 2000] as appropriate to this study. The complete criteria are available at ***http://www.eortc.be/recist/documents/RECISTGuidelines.pdf*** ~~http://www3.oup.co.uk/jnci/extra/920205.pdf~~.

Section 6.7.2. Hematology, serum chemistry and urine tests

Specification for Germany: An additional blood sample might be required for HIV test. Collect of the sample will be performed according to standard local practice. For more information, please refer to Section 13.2.

Section 11.2.3. Immunogenicity endpoints

This is explained in Section ***6*** ~~7~~ and is on the basis of:

Section 13.2. ***German specific requirements***

Concerning the HIV test

Upon request from Paul-Ehrlich-Institut (Germany), HIV test is mandatory to assess the HIV status (positive - negative) of patients in Germany only. Testing will be performed during the screening phase of the study and according to local standard practice. Patients with known HIV-positive status or positive for HIV as per testing will not be eligible for entry in the study. Patients for which the test is positive will be managed according to standard local clinical practice. Patients with known HIV-positive status do not need to be retested. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment.

Table 6 "List of study procedures for Cycle 1 (including screening)" has been adapted to include this HIV test.

Throughout the document

Orthographic and grammatical typos have been corrected. For a better readability, these corrections have not been highlighted in the body of the protocol.

GlaxoSmithKline Biologicals	
Clinical Research & Development	
Protocol Amendment 3	
eTrack study number and abbreviated title	112406 (NYESO1-AS15-MEL-001 (MET))
EudraCT number	2010-020663-20
Title	Study of GSK2241658A Antigen-Specific Cancer Immunotherapeutic in patients with unresectable and progressive metastatic cutaneous melanoma
Detailed title:	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1- positive unresectable and progressive metastatic cutaneous melanoma
Amendment number:	Amendment 3
Amendment date:	03 September 2014
Co-ordinating author:	PPD, PhD, *(Scientific writer, Cancer Immunotherapeutics)*
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The background and rationale for the changes made to the protocol as a consequence of Amendment 3 are described below:

The enrolment of new patients in the study, the follow-up of the patients who discontinued the study treatment due to disease progression or due to other reasons, or patients in the 1 year follow-up following the completion of the study treatment will be stopped given the fact that:

- the detailed analysis of the MAGRIT study (A double-blind, randomized, placebo-controlled Phase III study to assess the efficacy of recMAGE-A3 + AS15 Antigen-Specific Cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive Non-Small Cell Lung Cancer) showed the absence of treatment effect in any of the primary, secondary, or exploratory analyses. All aspects of the MAGRIT study have been carefully assessed, and unfortunately these investigations failed to identify a root cause for the lack of efficacy of the MAGE-A3 ASCI in NSCLC.
- a comprehensive review of the MAGE-A3 ASCI Phase III results, together with all other available clinical and laboratory data in early clinical studies with various recombinant proteins, including NY-ESO-1, tested in different diseases and settings suggests that the anticancer activity of these ASCI, all based on the recombinant protein combined with adjuvant system AS15 technology may well be very limited.
- the balance of anticipated benefits and apparent risks associated with NY-ESO-1 + AS15 ASCI continues to be acceptable following the ongoing systematic review of safety data. Also, no additional safety data will be collected, except for patients who continue treatment.

By stopping the active follow-up, the patients will not be further exposed to unnecessary study related procedures.

Although GSK has no reasonable expectation of a population benefit from the treatment, GSK cannot exclude that some patients in the NYES01-AS15-MEL-001 study may benefit from this treatment on an individual basis. Therefore, patients in the study and who are currently still receiving the study treatment, will be offered the option to continue treatment if they wish to do so, following discussion with their treating physician.

In the best interest of patients, blood drawings which were planned for protocol research purposes (i.e. PBMCs, serum collection) will not be performed anymore. Blood drawing for safety monitoring as per protocol will continue.

By default, for each biological sample already collected in the scope of this study and not tested yet, testing will not be performed except if a scientific rationale remains relevant. In this case, testing will be done in compliance with the protocol and ICF signed by the patient.

In the following sections, amended text has been included in *bold italics,* while deleted text is marked by ~~strikethrough~~.

Sponsor signatory	**Frédéric Lehmann, MD** ***Vice President, Head of Immunotherapeutics Cancer Incubator*** ~~Vincent Brichard, MD, PhD (*Senior Vice President, Head of Immunotherapeutics*)~~

SYNOPSIS

Rationale for the study and study design

1. Need for new therapeutic approaches for metastatic melanoma

The Final Container (per human dose) is composed of 420 µg of CpG7909 oligonucleotide ***(see note below),*** which will be co-lyophilized with the 300 µg recNY-ESO-1 protein and the liquid AS01B-4 adjuvant, which contains 50 µg of QS21 (a saponin molecule) and 50 µg of Monophosphoryl lipid A (*MPL*®), in a liposome formulation.

[Note: The concentration in CpG7909 depends on the lot used –Certain lots are labeled as containing 420 µg CpG, whereas other lots will be labeled as 380 µg CpG. This difference in how the CpG content is noted is due to the fact that a different analytical method to determine the content of the CpG was applied to more recent lots and the change in the content designation from 420 to 380 µg is a result of a recalculation. The actual content of the CpG in the lots is the same (within the allowable assay variation).]

2. Rationale for using recNY-ESO-1 protein combined with AS15 Adjuvant System

GSK recently reported the negative results of the MAGRIT study. The detailed analysis of MAGRIT showed the absence of treatment effect in any of the primary, secondary, or exploratory analyses. All aspects of the MAGRIT study have been carefully assessed, and unfortunately these investigations failed to identify a root cause for the lack of efficacy of the MAGE-A3 ASCI in NSCLC.

Furthermore, a comprehensive review of the MAGE-A3 ASCI Phase III results, together with all other available clinical and laboratory data in early clinical studies with various recombinant proteins, including NY-ESO-1, tested in different diseases and settings suggests that the anticancer activity of these ASCI, all based on the recombinant protein combined with adjuvant system AS15 technology may well be very limited.

In light of this, GSK has decided to stop further development of the recombinant protein adjuvanted portfolio as a standalone treatment for cancer patients.

Objectives

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.

Study design

- Duration of the study: The duration of the ASCI treatment period (from first visit to the concluding visit) will not last longer than 49 months for any patient, comprising the entire period of immunization and the concluding visit. The duration of the follow-up will be one year after the concluding visit. The total duration including follow-up is approximately 5 years.

As of Amendment 3, all active follow-up visits and procedures will be stopped. Post-study AEs/SAEs will continue to be reported as described in Section 9.3.1.

Endpoints

Secondary

- Progression-free survival (PFS).

PFS is defined as the time from first treatment to either the date of ***first*** disease progression ***(PD or SPD)*** or the date of death (for whatever reason), whichever comes first. Patients alive and without disease progression are censored at the date of the last visit/contact.

- The duration of response for patients with CR, PR or Stable Disease (SD) status.

The duration of the response is defined as time from first objective response or SD evaluation to first PD assessment ***or death***.

Immunogenicity endpoints

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.

- Immunogenicity of the NY-ESO-1 ASCI treatment will be evaluated at different timepoints.

On the basis of:

1. The anti-NY-ESO-1 ~~and anti-CPG7909~~ humoral antibody concentration and response.
2. The anti-NY-ESO-1 cellular (T-cell) response.

1. INTRODUCTION

1.2.2. The AS15 Adjuvant System

Per 0.5 mL dose, the liquid Adjuvant System AS01B-4 contains 50 µg of QS21 and 50 µg of Monophosphoryl lipid A (*MPL*®), in a liposome formulation. The immunostimulatory nucleotide CpG7909 is included ***[***420 µg per dose ***(see note in Section 5.1.1)]*** to enhance the immune response.

1.3.2.2 AS15 Adjuvant System

GSK recently reported the negative results of the MAGRIT study. The detailed analysis of MAGRIT showed the absence of treatment effect in any of the primary, secondary, or exploratory analyses. All aspects of the MAGRIT study have been carefully assessed, and unfortunately these investigations failed to identify a root cause for the lack of efficacy of the MAGE-A3 ASCI in NSCLC.

Furthermore, a comprehensive review of the MAGE-A3 ASCI Phase III results, together with all other available clinical and laboratory data in early clinical studies with various recombinant proteins, including NY-ESO-1, tested in different diseases and settings suggests that the anticancer activity of these ASCI, all based on the recombinant protein combined with adjuvant system AS15 technology may well be very limited.

In light of this, GSK has decided to stop further development of the recombinant protein adjuvanted portfolio as a standalone treatment for cancer patients.

2. OBJECTIVES

2.2 Secondary Objectives

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.

2.3 Translational research objectives

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.

3. STUDY DESIGN OVERVIEW

3.3 Duration of study participation

As of Amendment 3, all active follow-up visits and procedures will be stopped. Post-study AEs/SAEs will continue to be reported as described in Section 9.3.1.

Table 1: Overview of ASCI administrations, tumor and immunological evaluations [1]

Cycle no.			***Cycle 1***							***Cycle 2***							
Interval between ASCI administrations (admin.)			**2 weeks**							**3 weeks** *Continue treatment if CR or PR or SD or SPD at each TE*							
Visit no.	Screening		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Weeks after first ASCI admin. (w)	w-6 to w-1		0	2	4	6	8	10	12	14	17	20	22	23	26	29	31
recNY-ESO-1 + AS15 admin no.			**1**	**2**	**3**	**4**	**5**	**6**		**7**	**8**	**9**		**10**	**11**	**12**	
Tumor evaluation (TE)	**X**								**X**				**X**				**X**
Anti-NY-ESO-1 ~~and anti-CpG7909~~ Ab response			**X**		**X**		**X**	**X**	**X**							**X**	
Anti-NY-ESO-1 T cell responses			**X**					**X**								**X**	
Cycle no.	***Cycle 3***					***Cycle 4***											
Interval between ASCI admin.	**6 weeks**					**3 months**				**6 months**				**Concluding Visit**			
	Continue treatment if CR or PR or SD or SPD at each TE																
Visit no.	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*			
Week (w), month (m) or year (y) after first ASCI admin.	33w	39w	45w	51w	54w	V19 +3m	V21 +3m	V22 +3m	V23 +3m	V24 +6m	V25 +6m	V26 +6m	V27 +6m	V28 + 30 days			
recNY-ESO-1 + AS15 admin no.	**13**	**14**	**15**	**16**		**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**				
Tumor evaluation (TE)					**X**		**X**		**X**	**X**	**X**	**X**		**X**			
Anti-NY-ESO-1 ~~and anti-CpG7909~~ Ab response				**X**			**X**		**X**	**X**	**X**	**X**		**X**			
Anti-NY-ESO-1 T cell responses				**X**			**X**		**X**	**X**	**X**	**X**		**X**			

	~~*Follow-up*~~			
~~**Visit no.**~~				
~~**Months (m) after Concluding visit**~~				
~~Tumor evaluation~~				
~~Anti-NY-ESO-1 and anti-CpG7909 Ab response~~				
~~Anti-NY-ESO-1 T cell responses~~				

1. Abbreviations: Ab - Antibody; Admin - Administration; CR - Complete Response; m = Month (Month, as calculation convention: 1 month is defined as 4 weeks i.e. 28 days during the treatment phase); PR - Partial Response; SD - Stable Disease; SPD - Slow Progressive Disease; TE - Tumor Evaluation; w - Week; y - Year.
2. ***In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administration" rather than by the "weeks after first study treatment administration"***

4. STUDY POPULATION

4.3 Exclusion criteria for enrolment

1. The patient has at any time received systemic chemotherapy, biochemotherapy, small molecules or anti-CTLA-4 monoclonal antibody for metastatic disease.

Note: Isolated limb perfusion or ***local electrochemotherapy***, as long as this was performed at least 4 weeks before first ASCI administration is authorized.

3. The patient received any cancer immunotherapy containing a NY-ESO-1 antigen or any cancer immunotherapy for his/her metastatic disease.

Note: Previous adjuvant treatment with interferon, anti-CTLA-4 monoclonal antibody or a cancer immunotherapeutic ("vaccine") containing a tumor antigen other than NY-ESO-1 ***or radiotherapy*** is allowed, if the last administration took place at least 8 weeks before the first ASCI administration.

5. STUDY PRODUCT ADMINISTRATION

5.1.1 Description of study product

- One vial containing 300 μg of the recNY-ESO-1 protein co-lyophilized with 420 μg of CpG7909;

[Note: The concentration in CpG7909 depends on the lot used –Certain lots are labeled as containing 420 μg CpG, whereas other lots will be labeled as 380 μg CpG. This difference in how the CpG content is noted is due to the fact that a different analytical method to determine the content of the CpG was applied to more recent lots and the change in the content designation from 420 to 380 μg is a result of a recalculation. The actual content of the CpG in the lots is the same (within the allowable assay variation).] (Amended 03 September 2014).

5.3.2 Administration

Table 2: Dosage and administration

Treatment	Dose	Administration	
		Timing	***Route and site***
recNY-ESO-1 + AS15 ASCI	1 dose corresponding to 300 μg of recNY-ESO-1 protein and ***380*** ~~420~~ μg CpG, reconstituted in AS01B-4	Cycle 1: q2w x 6 Cycle 2: q3w x 6 Cycle 3: q6w x 4 Cycle 4: q3m x 4 then q6m x 4	i.m. Deltoid or lateral region of the thigh, preferably with alternation on right or left side at each succeeding injection

5.5.1 Criteria for postponement of ASCI administration

3. ***Any other medical reason that would expose the patient to an unacceptable risk, at the investigator's discretion.***

For these ***three*** ~~two~~ conditions, the entire program of study visits and ASCI administrations will be resumed as soon as the patient's condition allows or at the investigator's discretion (see Section 6.3.1). If the re-treatment criteria are not met within 4 weeks (3 months during and after Cycle 4) after the scheduled treatment date, the patient must be withdrawn from the study treatment and the procedures detailed for the Concluding Visit are to be carried out (see Section 6.5.4).

5.5.2 Criteria for permanent stopping of ASCI administration

For patients whose treatment is discontinued prematurely during the study, the procedures of the Concluding Visit (see Section 6.5.4) will be carried out a minimum of 30 days after the last ASCI administration and - if possible - before the initiation of any other treatment. ~~These patients should enter the follow-up period~~.

~~Patients discontinuing therapy prematurely in the absence of progression should not, if possible, receive any other cancer treatment before their disease progresses, unless it is clearly not in the best interest of the patient. Every effort will be made by the investigator to ensure documentation of the date of progression.~~

After disease progression, the choice of subsequent treatment will be at the discretion of the treating physician; the patient will be taken off study treatment and the investigator will document any further anti-cancer treatment administered ~~and assess the patient's best clinical response to second-line treatment.~~

6. STUDY ASSESSMENT AND PROCEDURES

6.3.1 Attendance of study visits

Table 3 Permitted deviations from stipulated dates of visits

Cycle 1:	± 3 calendar days
Cycle 2:	± 3 calendar days
Cycle 3:	± 4 calendar days
Cycle 4:	± 7 calendar days
~~Follow-up period:~~	~~± 14 calendar days~~

Table 4: Example of postponement of study treatment dose for 3 weeks during Cycle 1 and the delay of the schedule induced

Note: This postponement will have a similar impact on the complete schedule of visits and dose administrations throughout the entire study treatment period ~~and for the follow-up period~~. Any further postponement(s) will induce additional delay(s) that must be added.

6.4 Outline of study procedures

The timing of scheduled assessments for the 4 ASCI administration cycles is shown in Table 6 to Table 9. ~~The procedures during follow-up are described in Table 10~~.

Table 6: List of study procedures for Cycle 1 (including screening)

Visit no.	Screening [a]	1	2	3	4	5	6	7
Intervals between study treatment administrations [hj]		***2 weeks***						
Weeks after first ASCI administration	-	0	2	4	6	8	10	12
NY-ESO-1 ASCI admin no.	-	1	2	3	4	5	6	
Informed consent	●							
Addendum to informed consent [i]		●	●	●	●	●	●	●
Biopsy for NY-ESO-1 expression	●							
Inclusion/exclusion criteria	●	●						
Medical history	●							
Clinical evaluations								
Physical examination [b]	●	●	●	●	●	●	●	
Imaging procedures	● [c]							● [c]
Tumor response assessment								●
Safety assessments								
(Serious) Adverse Events recorded [d]	●	●	●	●	●	●	●	●
Potential immune-mediated diseases (pIMDs) recorded		●	●	●	●	●	●	●
Laboratory assessments								
Pregnancy test [e]	●	●	●		●		●	
Urine chemistry tests [f]	●						●	
HIV status [g]	●							
Hematological tests [f]	●	●		●			●	
Blood chemistry tests [f]	●	●		●			●	
Autoimmunity tests [f]	●						●	
Coagulation tests [f]	●	●		●			●	
~~Serum sampling collection [g]~~								
~~PBMC collection [h]~~								
Criteria for permanent stopping or postponement of study treatment			●	●	●	●	●	
Recording of concomitant medication		●	●	●	●	●	●	●

Note: For patients who are found to be NY-ESO-1 negative, only the following procedures need to be performed for this protocol: Informed consent, tumor biopsy, NY-ESO-1 expression testing, inclusion and exclusion criteria. All other procedures should only be performed if clinically indicated and do not need to be recorded on the eCRF.

a Screening visit/period is to take place a maximum of 6 weeks before the first ASCI administration. However:

1 Urine and blood chemistry, hematological, coagulation and autoimmunity tests, within 3 weeks before the first study treatment administration;

2 Negative pregnancy test must be obtained within 1 week before the first study treatment administration for women of child-bearing potential;

3 Tumor biopsy can be obtained within 12 weeks before the first ASCI administration; in the rare case the analysis of the sample would give an inconclusive result, the patient may be asked to allow a new sample to be collected or to allow the use of an already preserved sample to repeat the test. A tumor biopsy performed in the context of institution standard practice or another research study may be used for the NY-ESO-1 testing after obtaining the Sponsor's agreement, provided this biopsy was preserved in RNAlater® and was taken no more than 12 weeks before the first administration of the study treatment.

4 Tumor imaging no more than 6 weeks before first ASCI administration. Imaging performed as part of institution standard clinical practice or in relation to another research study does not need to be repeated, provided this imaging was performed no more than 6 weeks before the first administration of the study treatment.

5 HIV test within 3 weeks before the first ASCI administration, mandatory in Germany only (refer to Section 13.2).

b Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit***). Recording of all changes in the patient's medical condition including all adverse events.

c Chest computer tomography (CT) scan, complete-abdomen CT scan and any other examination medically indicated to assess tumor dissemination. A contrast-enhanced CT scan or magnetic resonance imaging (MRI) of the brain is mandatory at the Screening Visit; while at subsequent visits it should only be performed in case of evocative neurological symptoms. Color photography or ultrasound of skin/node/subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will need to be sent to the site according to GSK PII management procedure.***

d Refer to Section 9.3. At the screening visit only SAEs related to study participation and GSK concomitant medication will be recorded.

e For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained at screening, before the first ASCI administration and after 2, 6 and 10 weeks of treatment (at Visits 1, 2, 4 and 6) to allow continued recNY-ESO-1 + AS15 administrations.

f Refer to Section 6.7.2.

~~g Serum collection: collect 2 x 5 mL blood in dry tubes. For anti-NY-ESO-1 and anti-CpG7909 antibody humoral immunity response.~~

~~h Whole blood for peripheral blood mononuclear cells (PBMC) collection: PBMC will be obtained from 150 mL of venous blood. PBMC will be used for the cellular immune response analysis. Heparinized tubes will be shipped to GSK Biologicals or another laboratory, according to the instructions given to the investigation site.~~

g HIV test is mandatory to determine the HIV status (positive - negative) of patients in Germany only. Patients with known HIV-positive status do not need to be re-tested. Patients tested for HIV prior to signature of the ICF for study participation do not need to repeat the test, given that it was performed within three weeks of enrolment (refer to Section 13.2).

h In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administrations" rather than by the "weeks after first study treatment administration".

I Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.

Table 7: List of study procedures for Cycle 2

Visit no.	8	9	10	11	12	13	14	15
Intervals between study treatment administrations[f]	***3 weeks***							
Weeks after first ASCI administration ~~h~~[f]	14	17	20	22	23	26	29	31
NY-ESO-1 ASCI admin no.	7	8	9		10	11	12	
Addendum to informed consent[g]	●	●	●	●	●	●	●	●
Clinical evaluations								
Physical examination [a]	●	●	●		●	●	●	
Imaging procedures [b]				●				●
Tumor response assessment				●				●
Safety assessments								
(Serious) Adverse Events recorded [c]	●	●	●	●	●	●	●	●
pIMDs recorded	●	●	●	●	●	●	●	●
Laboratory assessments								
Pregnancy test [d]	●	●	●		●	●	●	
Urine chemistry tests [e]	●		●				●	
Hematological tests [e]	●		●				●	
Blood chemistry tests [e]	●		●				●	
Autoimmunity tests [e]							●	
Coagulation tests [e]	●		●				●	
~~Serum sampling collection [f]~~								
~~PBMC collection [g]~~								
Criteria for permanent stopping or postponement of study treatment	●	●	●		●	●	●	
Recording of concomitant medication	●	●	●	●	●	●	●	●

a Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit).*** Recording of all changes in the patient's medical condition including all adverse events.

b At Visits 11 and 15: chest, complete-abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will be sent to the site according to GSK PII management procedure.***

c Refer to Section 9.3.

d For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained before each ASCI treatment (at Visits 8-10 and 12-14) to allow continued recNY-ESO-1 + AS15 administrations.

e Refer to Section 6.7.2.

~~f Serum collection: collect 2 x 5 mL blood in dry tubes. For anti-NY-ESO-1 and anti-CpG7909 antibody humoral immunity response.~~

~~g Whole blood for PBMC collection: PBMC will be obtained from 150 mL of venous blood. PBMC will be used for the cellular immune response analysis. Heparinized tubes will be shipped to GSK Biologicals or another laboratory according to the instructions given to the investigation site.~~

f In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administrations" rather than by the "weeks after first study treatment administration".

g ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

Table 8: List of study procedures for Cycle 3

Visit no.	16	17	18	19	20
Intervals between study treatment administrations [g]	***6 weeks***				
Weeks after first ASCI administration [g]	33	39	45	51	54
NY-ESO-1 ASCI admin no.	13	14	15	16	
Addendum to informed consent [h]	●	●	●	●	●
Clinical evaluations					
Physical examination [a]	●	●	●	●	
Imaging procedures [b]					●
Tumor response assessment					●
Safety assessments					
(Serious) Adverse Events recorded [c]	●	●	●	●	●
pIMDs recorded	●	●	●	●	●
Laboratory assessments					
Pregnancy test [d]	●	●	●	●	
Urine chemistry tests [e]	●	●	●	●	
Hematological tests [e]	●	●	●	●	
Blood chemistry tests [e]	●	●	●	●	
Autoimmunity tests [e]				●	
Coagulation tests [e]	●	●	●	●	
~~Serum sampling collection [f]~~					
~~PBMC collection [g]~~					
Criteria for permanent stopping or postponement of study treatment	●	●	●	●	
Recording of concomitant medication	●	●	●	●	●
Conclusions for main analysis [i]					●

a. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit). Recording*** of all changes in the patient's medical condition including all adverse events.

b. At Visit 20: chest, complete-abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will be sent to the site according to GSK PII management procedure.***

c. Refer to Section 9.3.

d. For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained before each ASCI treatment (at Visits 16-19) to allow continued recNY-ESO-1 + AS15 administrations.

e. Refer to Section 6.7.2.

~~f. Serum collection: collect 2 x 5 mL blood in dry tubes. For anti-NY-ESO-1 and anti-CpG7909 antibody humoral immunity response.~~

~~g. Whole blood for PBMC collection: PBMC will be obtained from 150 mL of venous blood. PBMC will be used for the cellular immune response analysis. Heparinized tubes will be shipped to GSK Biologicals or another laboratory, according to the instructions given to the investigation site.~~

f Main analysis will be performed when all patients have either completed the treatment up to Cycle 3 or have withdrawn from study treatment.

g ***In case a study visit needs to be delayed for any reason, the date for the visits following the delayed visit will be determined by the "intervals between study treatment administrations" rather than by the "weeks after first study treatment administration".***

h ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

(Amended 03 September 2014)

Table 9: List of study procedures for Cycle 4

Visit no.	21	22	23	24	25	26	27	28	29
Date of Visit	V 19 + 3 m	V 21 + 3 m	V 22 + 3 m	V 23 + 3 m	V 24 + 6 m	V 25 + 6 m	V 26 + 6 m	V 27 + 6 m	V 28 + 30 days [a]
NY-ESO-1 ASCI admin no.	17	18	19	20	21	22	23	24	Concluding visit [b]
Addendum to informed consent [h]	●	●	●	●	●	●	●	●	
Clinical evaluations									
Physical examination [c]	●	●	●	●	●	●	●	●	●
Imaging procedures [d]		●		●	●	●	●		●
Tumor response assessment		●		●	●	●	●		●
Safety assessments									
(Serious) Adverse Events recorded [e]	●	●	●	●	●	●	●	●	●
pIMDs recorded	●	●	●	●	●	●	●	●	●
Laboratory assessments									
Pregnancy test [f]	●	●	●	●	●	●	●	●	
Urine chemistry tests [g]		●		●	●	●	●	●	●
Hematological tests [g]	●	●	●	●	●	●	●	●	●
Blood chemistry tests [g]	●	●		●	●	●	●	●	●
Autoimmunity tests [g]		●		●	●	●	●	●	●
Coagulation tests [g]		●		●	●	●	●	●	●
~~Serum sampling collection~~ [h]									
~~PBMC collection~~ [i]									
Criteria for permanent stopping or postponement of study treatment	●	●	●	●	●	●	●	●	
Recording of concomitant medication	●	●	●	●	●	●	●	●	●
Study conclusion									●

m = month, as calculation convention: 1 month is defined as 4 weeks i.e. 28 days during the treatment phase.

a. The procedures of the concluding visit are also to be carried out when a patient is withdrawn from study treatment.
b. For patients who have withdrawn because of disease progression, any resected tumor can be sent to GSK for analysis of the gene profile and other appropriate tests upon signature of the adequate ICF by the patient (refer to Section 12.2).
c. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers ***(only during the tumor assessment visit).*** Recording of all changes in the patient's medical condition including all adverse events.
d. At Visits 22, 24, 25, 26, 27 and 29: Chest, complete-abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory and must be performed within 2 weeks before the corresponding ASCI administration/visit. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions must also be performed ***if applicable. If a photography presents a characteristic area rending possible the patient identification, photography will not be loaded and will be sent to the site according to GSK PII management procedure.***
e. Refer to Section 9.3.
f. For women of child-bearing potential only; urine or blood test according to local practice; a negative result must be obtained before each ASCI treatment (at Visits 21-28) to allow continued recNY-ESO-1 + AS15 administrations.
g. Refer to Section 6.7.2.
~~h. Serum collection: collect 2 x 5 mL blood in dry tubes. For anti-NY-ESO-1 and anti-CpG7909 antibody humoral immunity response.~~
~~i. Whole blood for PBMC collection: PBMC will be obtained from 150 mL of venous blood. PBMC will be used for the cellular immune response analysis. Heparinized tubes will be shipped to GSK Biologicals or another laboratory, according to the instructions given to the investigation site.~~
h. ***Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 3. This only has to be done once. The study will continue only with patients from the active treatment group who will decide to stay in the study. Signature of the ICF addendum will be documented in the eCRF.***

~~Table 10: List of study procedures during follow-up~~

~~Visit No.~~	~~F1~~	~~F2~~	~~F3~~	~~F4~~
~~Months after Concluding Visit [a]~~				
~~Clinical evaluations~~				
~~Physical examination [b]~~				
~~Imaging procedures [c]~~				
~~Tumor response assessment~~				
~~Safety assessments~~				
~~SAE related to study participation and GSK concomitant medication [d]~~				
~~pIMDs recorded~~				
~~Recording of pregnancy~~				
~~Laboratory assessments~~				
~~Autoimmunity tests [e]~~				
~~Serum sampling collection [f]~~				
~~PBMC collection [g]~~				
~~Study follow-up conclusion~~				

~~b. Months are defined here as calendar months.~~
~~c. Complete physical examination. Superficial cutaneous lesions must be measured by use of a millimeter ruler or calipers. Recording of all changes in the patient's medical condition including all adverse events.~~
~~d. Chest, complete abdomen CT scans, and any other examination medically indicated to assess tumor dissemination are mandatory and must be performed within 2 weeks before the corresponding ASCI administration/visit. Brain contrast-enhanced CT scan (or MRI) should be performed only in case of evocative neurological symptoms. Color photography or ultrasound of skin / node / subcutaneous lesions must also be performed.~~
~~e. Refer to Section 9.3.~~
~~f. Refer to Section 6.7.2.~~
~~g. Serum collection: collect 2 x 5 mL blood in dry tubes. For anti-NY-ESO-1 and anti-CpG7909 antibody humoral immunity response.~~
~~h. Whole blood for PBMC collection: PBMC will be obtained from 150 mL of venous blood. PBMC will be used for the cellular immune response analysis. Heparinized tubes will be shipped to GSK Biologicals or another laboratory, according to the instructions given to the investigation site.~~

6.5.2.1 NY-ES0-1 expression screening of tumor lesions

- The tumor tissue has been preserved in ***RNAlater®*** ~~RNAlater~~ immediately after the surgery and the amount of tissue available must comply with the protocol requirements (see the SPM).

6.5.2.6 Tumor imaging and assessment

In order to enroll a patient in the trial, this tumor imaging should confirm that the patient is not presenting visceral metastasis. Color photography or ultrasound of skin/node/subcutaneous lesions must also be performed ***if applicable.***

~~Source documentation that is considered necessary by GSK Biologicals for efficacy or safety evaluation such as but not limited to pictures, CT-scans, histology, laboratory reports will be collected and upon request sent to GSK Biologicals for review, ensuring that patient confidentiality is maintained.~~

6.5.3.4. Blood sampling for safety or immune response assessments

As specified in the lists of study procedures in Section 6.4, blood samples are to be taken during certain study visits. Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

As of Protocol Amendment 3, no more blood samples will be collected for research purpose, i.e. blood samples taken for humoral immunological response and PBMC collection. For each blood sample already collected in the scope of this study and not tested yet, testing will not be performed by default, except if a scientific rationale remains relevant. Blood samples for safety assessments will continue to be collected and analysed.

- ~~Serum sample - a volume of 2 x 5 mL of whole blood should be drawn from patients for anti-NY-ESO-1 and anti-CpG7909 humoral immune response analysis at each predefined timepoint. After centrifugation, serum samples should be kept at -20°C until shipment.~~
- ~~A volume of at least 150 mL of whole blood should be drawn from patients for PBMC analysis of cell-mediated immune (CMI) response at each predefined time point. The blood should be stored at the investigator's site at room temperature and it must not be centrifuged. Samples will be shipped at room temperature (20 to 25°C) to the designated laboratory for cell separation to be performed within 24 hours.~~

6.5.4.1 Patients who received all the scheduled doses

Chest CT scan, complete-abdomen CT scan, and any other examination medically indicated to assess tumor dissemination. A contrast-enhanced CT scan or MRI of the brain should only be performed in case of evocative neurological symptoms. Color photography or ultrasound of skin/node/subcutaneous lesions must also be performed ***if applicable.***

At the visit, the following procedures described above will be performed: physical examination; recording any AEs and SAEs, pIMDs, and concomitant medications; taking a urine sample for urine chemistry tests; taking a blood sample for hematological tests, blood chemistry tests, autoimmunity tests, coagulation tests. ~~, serum sampling for humoral immunity tests and PBMC collection for cellular immunity tests.~~

6.5.4.2. Patients withdrawn before the end of the scheduled doses

The following procedures will be performed no more than two weeks before this visit. If imaging has been performed less than ***6*** **4** weeks before, this need not be repeated:

- Chest CT scan, complete-abdomen CT scan, and any other examination medically indicated to assess tumor dissemination. A contrast-enhanced CT scan or MRI of the brain should only be performed in case of evocative neurological symptoms.

During the visit, the following procedures described above will be performed: physical examination including the recording of the ECOG performance status; recording any (serious) AEs, pIMDs and concomitant medications; taking a urine sample for urine chemistry tests; taking a blood sample for hematological tests, blood chemistry tests, autoimmunity tests, coagulation tests~~, serum sampling for humoral immunity tests and PBMC collection for cellular immunity tests~~.

~~6.5.4.2. Procedures during follow-up phase~~

~~Patients, including those having withdrawn prematurely before having received all the scheduled ASCI injections, will be requested to attend follow-up visits every 3 months for the first year after the concluding visit. See Table 10 for the list of study procedures during the follow-up period and Sections 6.7.2 and 6.7.3 for details of the laboratory assays and immunological readouts conducted during the follow-up period.~~

~~There are 2 exceptions to this schedule of follow-up visits:~~

- ~~Patients who have withdrawn from the study because of withdrawal of consent will not be contacted again;~~
- ~~Patients who were withdrawn from study treatment because of disease progression or who progressed during the post-treatment study period will not be asked to return for complete study follow-up visits. These patients will be followed for survival by means of bi-annual contacts (e.g., by phone). If a second-line anti-cancer treatment is administered, the patient's best clinical response to this will be assessed, e.g., during the patient's standard visits to the institution.~~

~~Note: Patients withdrawn from the study treatment because of disease progression who do not receive any other anti-cancer treatment should be followed according to the 3-monthly follow-up visits schedule.~~

~~Also the imaging procedures and response assessments are conducted 2 weeks before follow-up visits 2 and 4 rather than at the time of the visit.~~

6.5.4.3 Study conclusion

The study conclusion will be made at ~~follow-up visit~~ 4 ***the Concluding Visit***. ~~The procedures to be performed are the same as those described for the follow-up period (Section 6.5.5).~~

6.6.1.1.2 Methods of measurement

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline ~~and during follow-up~~.

6.6.1.2.3. Evaluation of best overall response

Summary for evaluation of ~~best~~ overall response

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR ***(or NA)***	No	CR
CR	Incomplete response / SD	No	PR
PR	Any response other than PD	No	PR
SD	Any response other than PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Not all evaluated	***Any response other than PD***	***No***	***NE***
Any response other than PD	***Not all evaluated***	***No***	***NE***
Any	Any	Yes	PD

Abbreviations: CR: complete response; PR: partial response; SD: stable disease; PD : progressive disease; NA: not applicable; NE: non-evaluable.

6.6.2.1.1. Definitions

All measurements should be recorded in metric notation by use of a ruler or calipers. Baseline evaluations should be performed as closely as possible to the start of treatment (could be performed at Visit 1) and never more than ***6*** 4 weeks before the start of the treatment. ***(Amended 03 September 2014).***

6.6.2.2.1. Baseline documentation of target and non-target lesions

All other lesions should be identified as non-target lesions and should also be recorded and evaluated at baseline ***as well as at each further visit including a tumor response assessment.*** They should be followed as "present" or "absent" ***at baseline and followed using the RECIST criteria at further visits. Independently to this follow-up assessment, any new lesion will be recorded as non-target lesion.***

6.6.5.2 Mixed response for disease not evaluable according to RECIST criteria

- The appearance of new lesions in otherwise PR status of the LD of target lesions will be classified as "PR with new lesion ~~or more~~.

6.7 Biological sample handling and analysis

As of Protocol Amendment 3, no more biological samples will be collected for research purpose. For each biological sample already collected in the scope of this study and not tested yet, testing will not be performed by default, except if a scientific rationale remains relevant. In this case, testing will be done in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below.

6.7.2 Hematology, Serum chemistry and urine tests

Table 11: Hematology, Serum Chemistry, Urine tests

Sample type	System	Component	Scale	Timing
HEMATOLOGY				
Blood (5 mL)	Whole blood	Hemoglobin Hematocrit Complete blood cell count Total and differential white blood cell count Platelets	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 21 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
BIOCHEMICAL VALUES				
Blood (5 mL)	Serum	Sodium Potassium Calcium Lactate dehydrogenase (LDH) Creatine kinase (CK) Total protein[4] Albumin[4]	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 21, 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
HEPATIC FUNCTION				
Blood (5 mL)	Serum	Aspartate aminotransferase (ASAT) Alanine aminotransferase (ALAT) Alkaline phosphatase Gamma-glutamyl transpeptidase (γGT) Serum bilirubin[4] Direct bilirubin[3,4]	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 21, 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
RENAL FUNCTION				
Blood (5 mL)	Serum	~~Microalbuminuria[4] (cf. urinalysis)~~ Blood urea nitrogen (BUN)[5] Creatinine	Quantitative	During screening Cycle 1: Visit 6 Cycle 2: Visits 10 and 14 Cycle 3: Visits 17 and 19 Cycle 4: Visits 22, 25, and 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment
COAGULATION				
Blood (5 mL)	Serum	Prothrombin Time (PT) Activated Partial Thromboplastin Time (APTT)	Quantitative	During screening Cycle 1: Visits 1, 3 and 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s)

Sample type	System	Component	Scale	Timing
AUTOIMMUNITY				
Blood (5 mL)	Serum	Anti-nuclear antibody (ANA) [1]	Quantitative	During screening Cycle 1: Visit 6 Cycle 2: Visit 14 Cycle 3: Visit 19 Cycle 4: Visits 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s) Follow-up Visits: F2, F4
PREGNANCY TESTING (MANDATORY) [2]				
Blood (5 ml) or urine according to local practice	Serum or urine	According to local practice	Ordinal	During screening Cycle 1: Visits 1, 2, 4, and 6 Cycles 2 - 4: prior to each ASCI administration
URINALYSIS				
Urine	Urine	Protein (test strip) Glucose (test strip)	Ordinal	During screening Cycle 1: Visit 6 Cycle 2: Visits 8, 10 and 14 Cycle 3: Visits 16 to 19 Cycle 4: Visits 22, 24 to 28 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s)
		pH (test strip) Erythrocyte (Red Blood Cells - test strip) Microalbuminuria[3,4] (densitometric evaluation of urinary sediment)	Quantitative	

1. ANA test will be carried out by immunofluorescence on HEPG2 cells. In case anti-nuclear antibodies are detected, further analysis will be performed by immunodot and/or enzyme-linked immunosorbent Assay (ELISA) to identify the auto-target antigen.
2. For women of child-bearing potential only.
3. ***It is mandatory to measure direct bilirubin/microalbuminurea, only when bilirubin/albumin testing reveals abnormal results.***
4. ***A quantitative or qualitative test can be performed at the investigator's discretion. In cases where positivea qualitative results are received, test a quantitative test also needs to be performed. It is sufficient to do only one test for protein in Urine, if this test is sensitive enough.***
5. ***Measurement of serum urea (azotemia) is allowed instead of measurement of BUN. The choice between these tests is left at the investigator's discretion. If measurements are in serum urea, they need to be converted to BUN. The conversion has to made according to the following formula: Urea [mg/dL]= BUN [mg/dL] * 2.14. BUN can be expressed in either in mg/dL or in mmol/L. If Serum Urea has been expressed in mmol/L, the conversion factor is 1 and as the formula is: SU (mmol/L) = BUN (mmol/L).***

6.7.3 Molecular, immunological and translational research read outs

Note that, as of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. No further blood samples for protocol research purposes will be collected.

Table 12 Overview of molecular and immunological read-outs and translational research

***NY-ESO-1* SCREENING (MANDATORY)**			
Test	**Sample Type**	**Laboratory**	**Timing**
***NY-ESO-1* expression testing** (see also Section 6.7.3.1)	Fresh tissue sample (in *RNAlater*® or equivalent)	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.
IMMUNOLOGICAL READ-OUTS (MANDATORY)			
Test	**Sample Type**	**Laboratory**	**Timing**
Humoral immunity (see Section 6.7.3.2.1) Anti-*NY-ESO-1* ~~and anti-CpG7909~~ antibody response	Serum (2 x 5 ml) [b]	GSK Biologicals or contracted lab [a]	Cycle 1: Visits 1, 3, 5 to 7 Cycle 2: Visit 14 Cycle 3: Visit 19 Cycle 4: Visits 22, 24 to 27 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s) Follow-up Visits: F1 to F4
Cellular immunity (see Section 6.7.3.2.2) Cellular (T-cell) response (including regulatory T-cell responses)	Blood (150 ml) [c]		Cycle 1: Visits 1 and 6 Cycle 2: Visit 14 Cycle 3: Visit 19 Cycle 4: Visits 22, 24 to 27 Concluding Visit: Visit 29 Concluding Visit for patients withdrawn from study treatment(s) Follow-up Visits: F2, F4
MANDATORY TRANSLATIONAL RESEARCH (SEE SECTION 7.2.1)			
Test	**Sample Type**	**Laboratory**	**Timing**
Gene profiling of the tumor	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d]	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.

OPTIONAL TRANSLATIONAL RESEARCH (SEE SECTION 7 EXCEPT 7.2.1)			
Test	**Sample Type**	**Laboratory**	**Timing**
Expression analysis of NY-ESO-1 and other tumor antigens (e.g., MAGE-A3, PRAME, LAGE-1, MAGE-C2 and WT1)	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d]	GSK Biologicals or contracted lab [a]	During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.
***Natural immunogenicity and* Antigen spreading**	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy). In case of additional tumor biopsy or tumor resection.
			May be performed on any of the blood samples taken for the humoral and cellular immunity tests
Gene profiling of the immune response	Blood (150 ml) [c]		May be performed on any of the blood samples taken for the cellular immunity tests (PBMC collection)
Proteomic profiling	Fresh tissue sample (in *RNAlater*® or equivalent) and FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy) or on any of the blood samples taken for the humoral and cellular immunity tests
~~Tumor~~ infiltrating lymphocytes (including T regulatory cells and other immune cellular entities)	Either fresh tissue sample (in *RNAlater*® or equivalent) or FFPE tissue (if available) [d]		During screening (initial biopsy). In case of additional tumor biopsy or tumor resection
DNA methylation status of NY-ESO-1, other antigens and predictive genes, in addition to immunoregulatory genes	Either fresh tissue sample (in *RNAlater*® or equivalent) or FFPE tissue (if available) [d], serum and blood [b, c]		During screening (initial biopsy) or in case of additional tumor biopsy or tumor resection (disease progression), or on any of the blood samples taken for the humoral and cellular immunity tests
Pharmacogenetics analyses	Tumor samples and blood		Preferably on blood collected at Visit 1. On tumor cells from screening or from additional lesions

[a] GSK Biologicals laboratory or a validated laboratory designated by GSK Biologicals.
[b] 2 x 5 ml of blood will be used for both anti-*NY-ESO-1* ~~and anti-*CpG7909*~~ antibody responses; and for the optional translational research (as applicable).
[c] A total of 150 ml of blood will be used for cellular immunological read-outs; and for the optional translational research (as applicable).
[d] FFPE tissue is only to be collected from patients with sufficient tumor material from the same lesion to provide a fresh sample and a FFPE sample. GSK Biologicals or a contracted laboratory will require a block of at least 7 mm^3 or 15 slides of 10 μm each.

6.7.3.2 Immunological read-outs

6.7.3.2.1. Antibody response to the ASCI antigens

Currently, testing of serum to assess antibody mediated immune responses has been performed for a subset of samples collected in this study. Further testing will only be performed if a scientific rationale remains relevant in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below.

The antibody response to the NY-ESO-1 antigen ~~and to CpG7909~~ will be assessed for all patients enrolled into the study.

Specific anti-NY-ESO-1 ~~and anti-CpG7909~~ antibodies ***pre-existing and*** induced by the recNY-ESO-1 + AS15 ASCI will be measured by ELISA or upgraded techniques, and the results will be provided in EU/mL or other appropriate units. This can be done using either the NY-ESO-1 recombinant protein or NY-ESO-1-derived peptides spanning the entire NY-ESO-1 sequence as coating antigen. A patient will be considered as seropositive if the antibody titer is superior or equal to the assay cut-off value and at least twice the patient's value at baseline.

6.7.3.2.2. Cellular immune response to the ASCI antigens

Currently, testing of PBMCs to assess cell-mediated immune responses has been performed for a subset of samples collected in this study. Further testing will only be performed if a scientific rationale remains relevant in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below.

1. PBMC processing and analysis

The cell-mediated immune (CMI) response ***pre-existing or*** induced by the ASCI administrations will be assessed for all patients enrolled into the study.

2. Cellular response to the recNY-ES0-1 + AS15 ASCI

So far, in contrast to serology, there is no standard or validated technique available to monitor the T-cell response induced by immunization with a recombinant protein in cancer patients. The techniques described in this section may be updated to the best available at the time of analysis. Dependent on the immunogenicity of the tumor-antigen targeted, the T-cell responses can either be measured directly *ex vivo* or will require a step of specific *in vitro* re-stimulation of the T-cells. The presence of specific cells ~~can~~ ***may*** be documented by measuring the production of ~~anti-inflammatory~~ cytokines, by measuring a cytolytic activity (e.g. by detection of Granzyme, Perforin or CD107a marker), specific activation markers upon antigen encounter or by tetramer staining using known T cell-epitopes using appropriate assays. The percentage of those T-cells can then be determined by ELISPOT or by Fluorescence-Activated Cell Sorter (FACS), after surface and/or intracellular cytokine staining ***(Amended 03 September 2014)***.

~~Regulatory CD4 T-cells (Tregs) that express the transcription factor Foxp3 have been shown to accumulate in the intratumoral areas of metastatic melanoma tumors, and may play a role in immune suppression of anti-tumor immune responses [Ahmadzadeh, 2008].~~

~~In a separate study, $CD4^+$ Tregs that were stimulated to proliferate by two NY-ESO-1 peptides, were identified in the blood of patients with metastatic melanoma [Vence, 2007]. Both NY-ESO-1 peptides could induce IL-10 secretion in these $CD4^+$ Tregs and a proportion of these cells also expressed high levels of Foxp3. The presence of regulatory T-cell populations, defined by flow cytometry markers such as $CD4^+$, $CD25^+$ and $Foxp3^+$ and/or the IL10 secreting regulatory T-cells will be assessed in terms of their frequency and functionality using PBMCs or whole blood, if available.~~

Briefly, the characterization of the T-cell response ~~will~~ ***may*** be performed as follows:

- ~~To assess regulatory T-cells (Tregs), the most updated techniques will be used, e.g. enumeration of Tregs using a panel of antibody against *e.g.* CD3, CD4, CD25, Foxp3, CD39, CTLA4, GITR, PD1, ICOS, and OX40; enumeration of antigen-specific Tregs using activation markers specifically expressed on antigen-specific Treg (e.g. CD 69, PD-1) or in association to HLA Class II tetramers. The antigen specificity of Tregs can be assessed with epitopes from the NY-ESO-1 or from control antigens such as tetanus toxoid antigen or CMVpp65 (the most immunogenic protein of cytomegalovirus). The impact of Treg depletion on antigen specific proliferation and cytokine secretion, as well as IL10 or other suppressive cytokines secretion in immune response to various antigens can also be assessed. The type and number of read-outs will depend on the amount of PBMCs available for this.~~

The NY-ESO-1 cellular response will be assessed before immunization (to determine baseline values) and at several time points in each cycle of immunization ~~and twice during follow-up~~ (see Table 6 to Table 9).

7. TRANSLATIONAL RESEARCH

As of Amendment 3, the decision was taken not to perform further testing on biological samples already collected in this study by default, except if a scientific rationale remains relevant. In case testing would be performed in the future, testing will be done in compliance with the protocol and the ICF signed by the patient as described in the sub-sections below.

This section provides details on the translational research that may be done on the biological samples collected during this study. These different tests ***may*** ~~will~~ only be performed on biological samples from patients who voluntarily gave their consent to these specific assessments on the ICF, unless specified as mandatory for participation in the clinical study (see Section 7.2.1).

7.3 *Natural immunity and* antigen spreading induced by the recNY-ES0-1 + AS15 ASCI

On the basis of these findings, we will assess whether administration of the recNY-ESO-1 + AS15 ASCI is able to induce antibodies and T-cells against other tumor antigens that are co-expressed by the patient's tumor (e.g. MAGE-C2, MAGE-A3, LAGE-1, WT1, PRAME) in addition to tissue differentiation or overexpressed tumor antigens such as, for instance, Melan-A/MART-1 or gp100. The expression of these tumor antigens in the tumor presenting at baseline or in any progressive lesion(s) ***may*** ~~will~~ be assessed ***as well*** by quantitative RT-PCR or any updated technique at the time of the analysis. Validation of tumor antigen expression (including NY-ESO-1) at the protein level may also be performed by IHC or other proteomic analytical method.

7.4 Gene profiling of immunological response to the recNY-ES0-1 + AS15 ASCI

No additional blood sampling is needed as this gene profiling of the immune response may be performed on any of the blood samples collected for the analysis of the ***humoral or*** cellular immune response to the ASCI ~~(PBMC collection)~~. Gene expression profiling will be performed on total RNA extracted from whole blood or from purified populations ($CD4^+$ T-cells, $CD8^+$ T-cells, antigen-presenting cells) from PBMC by hybridization to a gene expression chip with a locked set of genes (e.g., Affymetrix or other commercial chips) ***or by the use of epigenetic markers (e.g. methylation).*** Further gene expression profiling and validation may be performed by Q-RT-PCR.

7.5 Proteomic profiling

Using ***appropriate*** ~~standard~~ techniques the serum or tumor proteome may be analyzed ~~by differential proteomics.~~ This will identify useful bio-markers for responsiveness to the NY-ESO-1 antigen.

7.7 DNA promoter methylation analysis of NY-ESO-1 and other tumor antigens

In addition, methylation status of regulatory regions of genes encoding transcriptional factors relevant in the regulation of immune response (such as *Foxp3, GATA-3, T-bet, ROR-γT* ***expressed by helper CD4 + T cells)*** might be analyzed. ***In the case of Foxp3 evaluation, the presence and characterization of circulating T regulatory cells may be evaluated in parallel or independently of the realization of these transcriptional profiling.***

9 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

9.1.3 Potential immune mediated diseases

Table 13 Examples of AEs to be recorded as pIMDs

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: e.g. non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy). • Narcolepsy	• Systemic lupus erythematosus and associated conditions • Systemic Scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome • Idiopathic inflammatory myopathies, including Dermatomyositis, Polymyositis, • Antisynthetase syndrome • Rheumatoid arthritis and associated conditions including Juvenile chronic arthritis and Still's disease) • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Alopecia areata • Lichen planus • Sweet's syndrome • Localised Scleroderma (Morphoea)
Liver disorders	**Gastrointestinal disorders**	**Endocrine disorders**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis.	• Inflammatory Bowel disease, including Crohn's disease, ulcerative colitis, microscopic colitis, ulcerative proctitis • Celiac disease • Autoimmune pancreatitis	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease • Polyglandular autoimmune syndrome • Autoimmune hypophysitis

Vasculitides	Blood disorders	Others
• **Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.** • **Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.**	• **Autoimmune hemolytic anemia** • **Autoimmune thrombocytopenia** • **Antiphospholipid syndrome** • **Pernicious anemia** • **Autoimmune aplastic anaemia** • **Autoimmune neutropenia** • **Autoimmune pancytopenia**	• **Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)** • **Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy)** • **Autoimmune myocarditis/cardiomyopathy** • **Sarcoidosis** • **Stevens-Johnson syndrome** • **Sjögren's syndrome** • **Idiopathic pulmonary fibrosis** • **Goodpasture syndrome** • **Raynaud's phenomenon**

~~Neuroinflammatory disorders~~	~~Musculoskeletal disorders~~	~~Skin disorders~~
~~Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy), and neuritis (e.g. optic neuritis)~~ ~~Multiple sclerosis (including variants)~~ ~~Transverse myelitis~~ ~~Guillain-Barré syndrome, (including Miller Fisher syndrome and other variants)~~ ~~Other demyelinating diseases (including acute disseminated encephalomyelitis)~~ ~~Myasthenia gravis (including Lambert-Eaton myasthenic syndrome)~~ ~~Non-infectious encephalitis/ encephalomyelitis~~ ~~Neuritis (including peripheral neuropathies)~~	~~Systemic lupus erythematosus~~ ~~Scleroderma (including, CREST syndrome and morphoea)~~ ~~Systemic sclerosis~~ ~~Dermatomyositis~~ ~~Polymyositis~~ ~~Antisynthetase syndrome~~ ~~Rheumatoid arthritis,~~ ~~Juvenile chronic arthritis, (including Still's disease)~~ ~~Polymyalgia rheumatica~~ ~~Reactive arthritis~~ ~~Psoriatic arthropathy~~ ~~Ankylosing spondylitis (including undifferentiated spondyloarthritides)~~ ~~Relapsing polychondritis~~ ~~Mixed connective tissue disorder~~	~~Psoriasis~~ ~~Vitiligo~~ ~~Raynaud's phenomenon~~ ~~Erythema nodosum~~ ~~Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)~~ ~~Cutaneous lupus erythematosus~~ ~~Alopecia areata~~ ~~Lichen planus~~ ~~Sweet's syndrome~~
~~Liver disorders~~	**~~Gastrointestinal disorders~~**	**~~Metabolic diseases~~**
~~Autoimmune hepatitis~~ ~~Primary biliary cirrhosis~~ ~~Primary sclerosing cholangitis~~ ~~Autoimmune cholangitis.~~	~~Crohn's disease~~ ~~Ulcerative colitis~~ ~~Ulcerative proctitis~~ ~~Celiac disease~~	~~Autoimmune thyroiditis (including Hashimoto thyroiditis)~~ ~~Grave's or Basedow's disease~~ ~~Diabetes mellitus type I~~ ~~Addison's disease~~

Vasculitides	Others
• ~~Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.~~ • ~~Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome, thromboangiitis obliterans (Buerger's disease), necrotizing vasculitis, allergic granulomatous angiitis, Henoch-Schonlein purpura, anti-neutrophil cytoplasmic antibody positive vasculitis, Behcet's syndrome, leukocytoclastic vasculitis.~~ • ~~Vasculitides secondary to other immune mediated diseases such as lupus vasculitis and rheumatoid vasculitis.~~	• ~~Autoimmune hemolytic anemia~~ • ~~Autoimmune thrombocytopenias~~ • ~~Antiphospholipid syndrome~~ • ~~Pernicious anemia~~ • ~~Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)~~ • ~~Uveitis~~ • ~~Autoimmune myocarditis/cardiomyopathy~~ • ~~Sarcoidosis~~ • ~~Stevens-johnson syndrome~~ • ~~Sjögren's syndrome~~ • ~~Idiopathic pulmonary fibrosis~~ • ~~Goodpasture syndrome~~

9.2.2 Pregnancy

The investigator, or his/her designee, will collect pregnancy information on any patient who becomes pregnant while participating in this study from the first ASCI administration ~~to the end of the follow-up period~~ ***until the Concluding Visit***.

9.3.1 Time period for detecting and recording adverse events, serious adverse events and pregnancies.

Study activity	Screening phase	Treatment phase			Concluding visit [1]	~~Follow up~~
Reporting of AEs and SAEs	ICF signature to Visit 1	Visit 1	Visit X (X= 2 to 28)	30 days after last ASCI administration	Visit 29	
Reporting of severe toxicity events						
Reporting of pIMDs and pregnancy						
Reporting of SAEs related to the study treatment						
Reporting of SAEs related to study participation and concurrent GSK medications, and reporting of any fatal SAEs, not due to disease progression						

1. Or the concluding visit for patients withdrawn from study treatment(s).

10 PATIENT COMPLETION AND WITHDRAWAL

10.1.2. Patient completion of the study

A patient will be considered to have completed the study when he/she has reached the end of all scheduled study visits. ~~The term "study activity" refers to physical visits and does not include follow-up contacts (i.e. phone contacts).~~

~~Follow-up after completion of ASCI administration should continue for 12 months after the concluding visit, unless the patient meets one of the criteria for earlier withdrawal (See Section 10.2.1).~~ Once the patient has been withdrawn from the study, the reason must be documented in the patient's medical records and eCRF.

10.2.1. Patient withdrawal from ASCI administration

In this case, a concluding visit will be performed with procedures described in Section 6.5.4 ~~and the patient will enter the follow-up period with follow-up visits for 1 year after completing the Concluding Visit (see Section 6.5.5 for a description of the follow-up procedures).~~

11 DATA EVALUATION CRITERIA FOR EVALUATION OF OBJECTIVES

This section describes the criteria for evaluation of objectives as initially planned. However, due to the Protocol Amendment 3, not all analyses described in this section will be performed. A detailed description of the analyses that will be done will be given in the Statistical Analysis Plan. The final analysis will occur when all patients have performed their Concluding Visit or have withdrawn from the study.

11.2.1 Clinical activity endpoints

- Progression-free survival (PFS).

PFS is defined as the time from first treatment to either the date of ***first*** disease progression ***(PD or SPD)*** or the date of death (for whatever reason), whichever comes first. Patients alive and without disease progression are censored at the date of the last visit/contact.

OS is defined as the time from first treatment until death. Patients alive at the time of analysis are censored at the time of the last visit/contact.

- The duration of response for patients with CR, PR or SD status.

The duration of the response is defined as time from first objective response or SD evaluation to first PD assessment ***or death***.

11.2.3 Immunogenicity endpoints

This is explained in Section 6 and is on the basis of:

1. The anti-NY-ESO-1 ~~and anti-CpG7909~~ humoral antibody concentration and response.
2. The anti-NY-ESO-1 ***specific*** cellular (T-cell) response.

11.4.2 According to protocol (ATP) cohort for analysis of immunogenicity

The ATP cohort for analysis of immunogenicity will include all evaluable patients (i.e., those meeting all eligibility criteria, who did not report major protocol deviation, who have complied with all the procedures defined in the protocol, who have received at least the first four study treatment administrations and have provided a result for immunogenicity evaluation within the 4 weeks following dose no. 4 and completed Visit 5). ~~The ATP cohort for analysis of immunogenicity will include all evaluable patients (i.e., those meeting all eligibility criteria, who have complied with all the procedures defined in the protocol, who have received at least the first four ASCI administrations and have completed Visit 5).~~ For each non-compliant patient, all data collected after protocol violation (i.e. treatment administered or blood sample collected too early or too late, concomitant medication, medical condition,…) will be eliminated from the ATP immunogenicity analyses.

11.5.1 Humoral immunogenicity

2. A seropositive patient is a patient whose anti-NY-ESO-1 ~~and anti-CpG7909~~ antibody titer is higher than or equal to the cut-off value.
3. Seroconversion in a patient is defined by the increase in antibodies (anti-NY-ESO-1 ~~and anti-CpG7909~~) from a titer below the cut-off level before the treatment to a titer above the cut-off level following treatment.

11.5.2 Cellular immunogenicity

A patient will be considered as a cellular-mediated immune responder if there is an increased amount of NY-ESO-1-specific T-cells after immunization as compared to the patient's baseline value. ~~These specific T-cells include the $CD4^+$ or $CD8^+$ T-cells producing cytokines and/or presenting cytolytic activity (or other, following the updated method of detection), as well as specific $CD4^+$ or $CD8^+$ T-cells presenting a particular phenotype (effector/memory).~~

The cut-off above which a patient will be considered ***to present NY-ESO-1 cellular immunogenicityas*** ~~an immune responder~~ will be determined ***and specified in the Statistical Analysis plan.***

~~Data from analysis of regulatory T-cells will be descriptive.~~

11.6 Conduct of analysis

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the ***statistical analysis plan and/or*** final study report.

11.6.1 Sequence of analysis

The final analysis will be performed when all patients have done their concluding visit or have withdrawn from the study. Due to Protocol Amendment 3, not all analyses described in this section will be performed. A detailed description of the analyses that will be done will be given in the statistical analysis plan.~~A main analysis will be performed when all patients have either completed the treatment up to Cycle 3 (Visit 20; week 54) or have withdrawn from study treatment.~~ A full clinical study report will be written including individual data listings.

~~A final analysis will be performed when all patients have either completed the treatment up to Cycle 4 or have withdrawn from study treatment, and will be reported in annexes to the study report~~.

~~Follow-up analysis will be performed when all patients have either completed the follow up or have withdrawn from study, and will be reported in annexes to the study report.~~

11.7 Statistical methods

Due to Protocol Amendment 3, not all analyses described in this section will be performed. A detailed description of the analyses that will be done will be given in the statistical analysis plan.

11.7.2 Analysis of clinical activity

- Kaplan-Meier curves will be estimated for TTF, PFS and OS. If study treatment failure does not occur before the patient's last study visit, then the time to study treatment failure will be censored at the date of the last visit. If progression does not occur before the patient's last study visit, then the time to progression will be censored at the date of the last visit. If the patient is withdrawn from the study treatment for other reasons than progression, then the time to study treatment failure or progression will be censored at the date of the last assessment of the patient. ***For OS, patients who are still alive at the time of the analysis will be censored at the date last known to be alive.***

11.7.3 Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for immunogenicity. Humoral and cellular responses will also be analyzed for the total treated cohort if more than 3 patients are excluded from the ATP cohort for immunogenicity. ~~In this total treated cohort analysis, patients without immunogenicity measurements after ASCI treatment will be treated as non immunological responders.~~

For each cohort, at each blood sampling time point ~~and for each antibody (anti-NY-ESO-1 and anti-CpG7909 antibodies)~~ for which results are available:

- ***Anti-NY-ESO-1 antibody s***eropositivity rate, humoral response rate and antibody geometric mean titers (GMTs) with 95% CIs ***will be measured or calculated***;
- Percentages of ~~seroconverted~~ patients ***presenting NY-ESO-1 immunogenicity scores above cut-off*** and cellular ~~(~~T-cell~~)~~ ***responses to NY-ESO-1*** ~~responders~~ with exact 95% CIs will be given***. Estimations of the cellular T cell precursor frequencies will be given if available.***
- In addition, individual data over time will be displayed graphically ~~and per timepoint.~~

Protocol Amendment 3 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	112406 (NYESO1-AS15-MEL-001 (MET))
IND number	14313
EudraCT number	2010-020663-20
Date of protocol amendment	Amendment 3 Final: 03 September 2014
Detailed Title	An open Phase I Study of immunization with the recNY-ESO-1 + AS15 Antigen-Specific Cancer Immunotherapeutic in patients with NY-ESO-1-positive unresectable and progressive metastatic cutaneous melanoma
Sponsor signatory	**Frédéric Lehmann, MD** ***Vice President, Head of Immunotherapeutics Cancer Incubator (Amended 03 September 2014)***
Signature	PPD
Date	19.09.14

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