A randomized, double blind, placebo controlled phase II trial to evaluate the safety and efficacy of recMAGE-A3 + AS15 ASCI in patieNts with MAGE-A3 pOsitive muscLe Invasive blAdder cancer after cystectomy

Acronym: MAGNOLIA

A European Association of Urology Research Foundation Randomized Phase II Clinical Trial

CONFIDENTIAL

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Sponsor:	EAU Research Foundation Mr. E.N. van Kleffensstraat 5 NL 6842 CV Arnhem PO Box 30016 6803 AA Arnhem The Netherlands Tel: + 31 26 3890677 Fax: +31 26 389 0679 E-mail: researchfoundation@uroweb.org www.uroweb.org
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Principal Investigator:	Peter F.A. Mulders, MD, PhD Dept of Urology, Radboudumc Geert Grooteplein 10, NL 6525 GA Nijmegen The Netherlands Telephone: +31 24 3613735 Email: peter.mulders@radboudumc.nl
Protocol writing committee:	Wim P.J. Witjes, MD, PhD, Arnhem Christien T.M. Caris, MSc, Arnhem
Study Management:	EAU Central Research Office Contact details: see EAU Research Foundation

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List of Abbreviations and Relevant Definitions

AE	Adverse Event: any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
ALAT	Alanin-aminotransferase
ASAT	Aspartate-aminotranferase
ASCI	Antigen Specific Cancer Immunotherapy
ATP	According To Protocol
BCG	Bacillus Calmette-Guérin
CA	Competent Authority
CIS	Carcinoma in Situ
CRF	Case Report Form
COPD	Chronic Obstructive Pulmonary Disease
CRO	Contract Research Organisation
CT	Computer Tomogram
CTCAE	Common Terminology Criteria for Adverse Events
CV	Concluding Visit
Cxr	Chest X-ray
CYP	Cytochrome P450
DFS	Disease Free Survival
DFSS	Disease Free Specific Survival
DMFS	Distant Metastases Free Survival
DNA	Desoxyribo Nucleic Acid
EAU RF	European Association of Urology Research Foundation
EAU CRO	EAU Central Research Office
EC	medical Ethics Committee
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FFPE	Formalin Fixed Paraffin Embedded
GCP	ICH Harmonised Tripartite Guideline E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) Step 5, adopted by CPMP July 1996. European Directives 2001/20/EC and 2005/28/EC
GSK	GlaxoSmithKline Biologicals, Rixensart, Belgium

HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethical Committee
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IUD	Intra Uterine Device
IUS	Intra Uterine System
IV	Intra Venous
IVU	IntraVenous Urogram
recMAGE-A3-ASCI	Recombinant Melanoma AntiGEn-A3 Antigen Specific Cancer Immunotherapy
MIBC	Muscle Invasive Bladder Cancer
MPL	Monophosphoryl Lipid A
MRI	Magnetic Resonance Imaging
NSCLC	Non Small Cell Lung Cancer
PI	Principal Investigator
pIMD	potential Immune-Mediated Disease
PS	Performance Status
RNA	RiboNucleic Acid
RT PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event: A serious adverse event is any untoward medical occurrence that at any dose: results in death; is life-threatening; requires inpatient hospitalisation or prolongation of existing hospitalization; results in persistent or significant disability or incapacity or is a congenital anomaly/birth defect.
SBIR	GSK Biologicals' system for randomization over the Internet
SUSAR	Suspected Unexpected Serious Adverse Reaction: A Serious Adverse Event that is both attributable to investigational product and unexpected.
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but

	referred to as a subsidising party. The sponsor for this clinical trial is EAU RF.
TNM	Tumor, Nodes and Metastases
TUR	TransUrethral Resection
ULN	Upper Limit of Normal
WHO	World Health Organisation

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1. STUDY SYNOPSIS

Study Title and number	A randomized, double blind, placebo controlled phase II trial to evaluate the safety and efficacy of recMAGE-A3 + AS15 ASCI in patients with MAGE-A3 positive muscle invasive bladder cancer after cystectomy A European Association of Urology Research Foundation Randomized Phase II Clinical Trial. EAU-RF 2010-01
Rationale	Currently, the standard treatment for localized muscle invasive bladder cancer is radical cystectomy. • **Many patients with muscle invasive bladder cancer will relapse after cystectomy** The 10-year disease-specific and overall survival of patients with organ confined (defined as < pT3a) is 72.9% and 49.1%, rapidly decreasing to 33.3% and 22.8% for non-organ confined disease (13). There is thus a clear medical need for an additional anti-tumoral treatment in this population. • **There is not enough evidence in favour of the routine use of adjuvant chemotherapy** From scientific evidence so far available, it is unclear whether immediate adjuvant chemotherapy or chemotherapy at the time of relapse is superior or if the two approaches are equivalent with respect to the end-point overall survival. • **MAGE-A3 is a factor of poor prognosis** The interest in recMAGE-A3 ASCI treatment is further reinforced by the possible link between MAGE-A3 expression and shorter survival (34)

	• **MAGE-A3 is tumor-specific, recMAGE-A3 ASCI treatment highly tolerated and shows promising Phase II results** Taking into account the tumor-specificity of MAGE-A3, the high tolerability of recMAGE-A3 + AS15 and the promising results from the Phase II clinical trials in melanoma and lung cancer, EAU RF proposes to initiate a randomized, placebo-controlled clinical Phase II trial with recombinant MAGE-A3 (recMAGE-A3) combined with the AS15 adjuvant in patients with muscle invasive bladder cancer with MAGE-A3 expression after cystectomy. This trial will assess whether adjuvant treatment with recMAGE-A3 + AS 15 ASCI after cystectomy is safe and effective and improves outcome of MAGE-A3 positive patients after cystectomy. The rationale of the MAGNOLIA study has been changed (see added section Study Synopsis below and Section 2.6.2).
Objectives	The primary objective of this Phase II study is to evaluate the clinical efficacy in terms of Disease Free Survival of recMAGE-A3 + AS 15 ASCI versus placebo in the overall population. Secondary objectives are: • To evaluate overall survival in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy • To evaluate Disease-free (DFS) in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy. • To evaluate Disease-free specific survival (DFSS) in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy.

	• To evaluate Distant metastasis-free survival (DMFS) in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy. • To evaluate the safety of recMAGE-A3 + AS15 ASCI in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy. • Translational research: i) To identify a gene signature predictive to recMAGE-A3+AS15 ASCI in MIBC. ii) To evaluate on exploratory basis a possible correlation between gene expression profile of the primary tumor and clinical efficacy of recMAGE-A3 + AS15 ASCI compared to placebo in terms of: 1. Disease-free Survival (DFS) 2. Overall survival iii) To evaluate expression of genes in a previously identified gene signature and evaluate their correlation with clinical efficacy of recMAGE-A3 + AS15 ASCI compared to placebo in terms of: 1. Disease-free Survival (DFS). 2. Overall survival. 3. Disease-free specific survival (DFSS) 4. Distant metastasis-free survival (DMFS). iv) To characterize the tumor microenvironment and lymphocyte infiltration in the primary tumor and its recurrence lesions As of Protocol Amendment 4.0, the primary and secondary objectives of the MAGNOLIA study will not be assessed as planned (see added section Study Synopsis below and Section 2.6.2).
Study Design &	This is a multicentre, prospective, randomized, placebo-controlled, parallel group, double-blind, trial to compare the efficacy and safety of recMAGE-

Intervention	A3 + AS15 ASCI intramuscular injections with Placebo intramuscular injections. The target was to enroll 273 patients to be randomly assigned to 2 treatment schedules in a 2:1 ratio, 2 patients randomized for recMAGE-A3 + AS15 ASCI versus 1 patient randomized for placebo, either directly after recovery from surgery, or after recovery from adjuvant chemotherapy. The treatment scheme consists of 5 doses administered at 3-week intervals followed by 8 doses administered at 3-month intervals for a total maximum duration of study treatment administration of 27 months. Data collection will be organised by Remote Date Entry on Electronic Case Report Forms (eCRF). Ten European countries are participating with a total of 50 centres. Sites contributed 0-28 screened patients in the recruitment period of 40 months. Enrolment in this study was competitive.
Study Population	Patients with histologically confirmed urothelial carcinoma of the bladder ($T_{2,3}$ N_0 or N_1 or N_2 and M_0 disease or Stage T_4 N_0 M_0 disease) which was MAGE-A3 positive, who were free of residual disease and free of metastases, were randomized a few days before first study treatment administration. At the time of putting the recruitment on hold on 23 September 2014 (see added section Study Synopsis below and Section 2.6.2), 83 patient out of the 273 initially foreseen were randomized at 29 sites.
Main study Endpoints	Primary endpoint is Disease-free Survival (DFS): Secondary endpoints are: • Overall Survival. • Disease-free specific survival (DFSS). • Distant metastasis-free survival (DMFS). • (Serious) Adverse events. • To evaluate impact of predictive gene signature on efficacy of treatment in terms of DFS and overall survival.

	• To evaluate Gene expression profiles of the primary tumor by microarray. As of Protocol Amendment 4.0, primary and secondary endpoints will not be assessed as planned (see added section Study Synopsis below and Section 2.6.2).
Nature and extent of the burden and risks associated with participation and risk-benefit	Given the situation of the patient who needs a cystectomy, the extra burden and risks associated with participation in the study is considered minimal and acceptable. During the first 3 months in the study, the number of patient visits is higher. This is due to the need for administration of treatment every 3 weeks. Hereafter, the number of visits and treatments is equal to what patients with these criteria is offered when they are treated in the standard way, which is watchful waiting after cystectomy. Possible extra procedures in this study depend on local routine practices. For example 3-monthly CT scans /MRI's can be extra if 6-monthly imaging procedures are considered standard routine practice (during treatment phase only). Also laboratory assessments and the questionnaire on smoking habits can be considered as extra. The risks related to the expected treatment outcome, quality and quantity of side effects of the study medication can be considered as acceptable (see Section 2.5.3). Benefit for the patient is that the patient is followed according to the latest standards and guidelines of treatment of MIBC and the patient has a chance on early detection of recurrence of disease.
Timing	As of Protocol Amendment 4.0, the timing of the MAGNOLIA study has been changed (see added section Study Synopsis below and section 2.6.2). The total duration of the study will be 7 years, with a recruitment phase of 40 months The duration of the treatment period will not exceed 108 weeks for any patient, comprising the entire period of treatment administration and excluding the concluding visit (Visit 14), provided there are no postponements. The concluding visit will take place at least 30 days after the patient's last dose of the study product. Treatment will not be possible anymore after 30 November 2016. The 2,5-year active follow-up period planned in the initial study protocol has been cancelled.

Protocol amendment 4.0	Upon release and analysis of the MAGRIT trial results, the rationale of the MAGNOLIA study has been changed. As of 23 September 2014, the recruitment was put on hold (sites were still allowed until 3 February 2015 to randomize patients that signed the informed consent or were in the screening process). GlaxoSmithKline Biologicals recently reported the negative outcome of the MAGE3-AS15-NSC-003 (ADJ) (109493 - EudraCT number: 2007-001283-73) MAGRIT study (A double-blind, randomized, placebo-controlled Phase III study to assess the efficacy of the recMAGE-A3 + AS15 Antigen-Specific Cancer Immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive Non-Small Cell Lung Cancer (53). The analysis of MAGRIT confirmed 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 Antigen-Specific Cancer Immunotherapeutic (ASCI) in NSCLC that could be corrected in ongoing studies, including the MAGNOLIA study. Furthermore, a comprehensive review of the Phase III results, together with all other available clinical and laboratory data with various recombinant proteins tested in different diseases and settings now suggests that the anticancer activity of this technology may well be limited to a subgroup of Stage III melanoma patients with a specific predictive gene signature (52). A few patients in early metastatic melanoma also appear to benefit from this type of treatment. Consequently, GSK Biologicals has decided to stop further development of recMAGE-A3 + AS15 as a standalone treatment for cancer patients. This decision is not motivated by any safety concern as confirmed by all Independent Data Monitoring Comittees (IDMCs) overlooking the ASCI trials. This decision implies that MAGE-A3 +AS15 CI will not be available for future treatment of bladder cancer patients which warrants a substantial amendment (4.0) of the MAGNOLIA study.

	As of Protocol Amendment 4.0, the recruitment will be stopped and the study population will be unblinded. For patients randomized to the placebo group, no further protocol visits will be performed except for the concluding visit and no further doses will be administered. As it cannot be excluded that one or more patients may benefit from this treatment on an individual basis, patients receiving active treatment (patients not on placebo, and patients that have not already completed or interrupted their MAGE-A3 ASCI treatment) will be offered the option to continue the administration of the study treatment until the last dose is administered or until recurrence, whichever comes first, or until the patient or the investigator decides to stop the study treatment. Therefore, the study will continue only with patients from the active treatment group who will decide to stay in the study. Treatment will not be possible anymore after 30 November 2016. During the treatment period, safety monitoring will continue as initially foreseen during the treatment period. In the best interest of the patient, additional biological samples for protocol research purposes (evaluation of the immune response, translational research) will not be taken nor collected. The primary and secondary objectives will not be assessed as planned. All clinical data collected in the study will be analysed descriptively. By default, for each biological sample already collected in the scope of this study and not tested yet, testing will only be done if a scientific rationale remains relevant despite the premature termination of the study. In that case, testing will be in compliance with the protocol and ICF signed by the patient. The immune response will not be evaluated anymore as the immune response to IP administration was confirmed in other ASCI trials, but did not show any correlation with the potential efficacy.

2. INTRODUCTION AND RATIONALE

2.1 Epidemiology of Bladder Cancer

In 2006, in Europe, an estimated 104,400 incident cases of bladder cancer were diagnosed, of which 82,800 were diagnosed in men and 21,600 in women. This represents 6.6% of the total cancers in men and 2.1% in women, with an estimated male-to-female ratio of 3.8 : 1. In men, bladder cancer was the fourth most common cancer. Bladder cancer resulted in 4.1% of total deaths for cancer in men and 1.8% of total deaths in women (1). At the initial diagnosis of bladder cancer, 70% of cases are diagnosed as non-muscle-invasive disease and 30% as muscle-invasive disease. Among patients treated with radical cystectomy because of muscle-invasive disease, 57% had muscle invasion at presentation, while 43% had been initially diagnosed with non-muscle-invasive disease that progressed despite organ-preserving treatment (2). Approximately one-third of patients diagnosed with muscle-invasive bladder cancer have undetected metastases at the time of treatment of the primary tumour (3), while 25% of patients submitted to radical cystectomy present with lymph node involvement at the time of surgery.

2.2 Standard Treatment of Bladder Cancer

Radical cystectomy is the standard treatment for localized muscle invasive bladder cancer in most countries of the Western Hemisphere (4,5). Radical cystectomy also includes the dissection of regional lymph nodes. There is a substantial amount of literature about the extent of lymphadenectomy. Yet, data regarding its clinical significance is controversial. In retrospective studies extended lymphadenectomy (removal of the obturator, internal, external, common iliac and presacral nodes as well as nodes at the aortic bifurcation) has been reported to improve survival in patients with muscle invasive bladder cancer (8). The curative value of lymph node dissection, however, is still unknown and a standardized lymph node dissection has yet to be defined (6-8).
In a comprehensive study regarding long-term results in 1,054 cystectomy patients, perioperative mortality was 3%, and early complications, defined as any complication within 3 months of surgery, were reported in 28% (9,10). Late morbidity is usually due to the type of urinary diversion. Early morbidity associated with radical cystectomy for non-muscle invasive disease (at high risk for disease progression) is similar and not less than that associated with muscle invasive tumours (11).

2.3 Survival Results of Standard Treatment

The outcome according to a multiinstitutional database of 888 consecutive patients undergoing cystectomy and lymphadenectomy for bladder cancer revealed a mean recurrence-free and bladder cancer specific survival of 58% and 66%, resp. at 5 years (12). The recurrence-free and overall survival in a large single centre study of 1,054 male and female patients was 68% and 66% at 5 years and 60% and 43%, at 10 years, respectively (5). In node positive patients, 10-year disease-specific and overall survival rates in another study have been reported to be 27.7% and 20.9% respectively (13). In this cohort, 10-year disease-specific and overall survival rates were 72.9% vs. 49.1% for organ confined (defined as < pT3a), and 33.3% vs. 22.8% for non-organ confined disease (13). In another study, 5-year recurrence-free survival was 76% in patients with pT1 tumours, 74% for pT2, 52% in pT3, and 36% in pT4 tumours (14). Tumour stage and nodal involvement are the only independent predictors of survival (15).
Local recurrence accounts for about 30% of relapses, whereas distant metastases are more common. Before the development of effective chemotherapy, patients with metastatic urothelial cancer rarely exceeded the median survival of 3-6 months (16). Chemotherapy increases overall survival in patients with relapse after cystectomy or unresectable disease.

2.4 Adjuvant Chemotherapy

The value of adjuvant chemotherapy for patients after radical cystectomy with pT3/4 and/or lymph node positive (N+) disease without clinically detectable metastases (M0) is under debate (17,18).

The benefits of chemotherapy in the adjuvant setting include:
• Chemotherapy is administered after accurate pathological staging
• Overtreatment in patients at low risk for micrometastases is avoided
• Compared to neoadjuvant chemotherapy, there is no delay in definitive surgical treatment, especially in patients not sensitive to chemotherapy.

The drawbacks of adjuvant chemotherapy are:
• Assessment of in-vivo chemosensitivity of the tumour is not possible
• Delay or intolerability of chemotherapy, due to post-operative morbidity
• Chemotherapy related morbidity and impact on quality of life.

There is not enough evidence in favour of the routine use of adjuvant chemotherapy (18, 24). To date, there have been only five published randomized trials of adjuvant chemotherapy (19-23) and one meta-analysis (24), with updated individual patient data from six trials and a total of only 491 patients for survival analysis.
Furthermore, all these trials are sub-optimal with serious deficiencies, such as low sample size (underpowered), use of substandard chemotherapy, early stopping of patient entry and flaws in design and statistical analysis, including irrelevant endpoints or a lack of recommendations concerning salvage chemotherapy for relapse or metastases (18). The data are not convincing enough to give an unequivocal recommendation for the use of adjuvant chemotherapy.
From the evidence so far available, it is unclear whether immediate adjuvant chemotherapy or chemotherapy at the time of relapse is superior or if the two approaches are equivalent with respect to the end-point overall survival. In recent trial updates, cisplatin-based combination chemotherapy was able to produce long-term disease-free survival, even in metastatic disease, albeit mainly in patients with lymph node metastases only and in good PS (25-27). Published trials of randomized adjuvant chemotherapy have used 3-4 cycles of CMV (cisplatin, methotrexate, vinblastine), CISCA (cisplatin, cyclophosphamide, and adriamycine), MVA(E)C (methotrexate, vinblastine, adriamycine or epirubicine, and cisplatin) and CM (cisplatin, methotrexate) (28).

2.5 recMAGE-A3 Antigen Specific Cancer Immunotherapy (recMAGE-A3 + AS15 ASCI)

The study investigational product is an Antigen-Specific Cancer Immunotherapeutic (ASCI) comprising the recombinant antigen ProtD-MAGE-A3/His (recMAGE-A3) and the GSK proprietary immunological adjuvant AS15.
Multiple phase I/II/III studies with the recMAGE-A3 to be used in this study have been performed or are in progress. Treatment with recMAGE-A3 was associated with clinical responses mainly in metastatic melanoma and NSCLC, including partial responses, some complete responses with significant long-term duration. In addition, the safety of this treatment was excellent, and an immunological response could be measured in the group of patients administered the adjuvanted recMAGE-A3 (29-32)
Since MAGE-A3 tumour antigen is expressed in approximately 40% (GSK Internal data) of patients with bladder cancer, the possibility that recMAGE-A3 + AS 15 ASCI may also be an efficient therapy in patients with bladder cancer needs to be explored.

2.5.1 MAGE-A3

MAGE genes and, particularly the gene coding for the MAGE-A3 protein, are silent in all normal adult tissues with the exception of testis and placenta, but are re-activated in many cancers including non-small-cell and small-cell lung carcinoma, head and neck squamous cell carcinoma, urothelial carcinoma of the bladder, oesophagus carcinoma and malignant melanoma. The MAGE-A3 protein is strictly tumor-specific, a finding supported by the fact that its expression is not detected by RT-PCR in any normal adult tissue with the exception of testis and placenta. However, the normal testis and placenta cells that express the gene do not bear molecules from the major histocompatibility complex on their surface and therefore do not present any MAGE-A3 epitope to the immune system.

The recMAGE-A3 protein used is a recombinant fusion protein produced in E. Coli, purified and freeze –dried in single-dose potions. For administration, the recMAGE-A3 protein is dissolved in a GSK proprietary immunostimulatory Adjuvant System immediately before injection.

2.5.2 recMAGE-A3 ASCI preclinical experience

The immune response induced by injections of the recombinant recMAGE-A3 protein formulated in different Adjuvant systems was investigated in the mouse model. The recMAGE-A3 protein was found to be weakly immunogenic by itself. However, in the presence of an adjuvant immunostimulant a robust immune response was induced, including MAGE-A3 –specific antibodies and T-cells. Immunized mice were protected against a challenge with tumor cells expressing MAGE-A3.
Extensive preclinical toxicity testing has been performed with the Adjuvant Systems that are used with recMAGE-A3, and with the various immunostimulants included in these Adjuvant Systems in combination with other cancer antigens (HER2/neu, P501) without any results that would raise concern about their safety in humans. See investigator’s brochure.

2.5.3 recMAGE-A3 ASCI clinical experience

In the terminated studies so far, a total of 1605 patients have received recMAGE-A3 alone or together with four different Adjuvant Systems, in a total of at least 10546 doses (cut-off date: April, 2010) (33). The AS15 Adjuvant System appeared to have the strongest and most robust

immunological response and was considered as having the highest potential to lead to improved clinical effect. Out of these 1605 patients, 1295 patients have received a total of some 7966 doses of the recMAGE-A3 + AS15 ASCI. In addition to this, it can be estimated that at least some 220 patients have been randomized to and received at least one dose of the recMAGE-A3 + AS15 ASCI in the two ongoing large randomized Phase III studies (in patients with NSCLC and melanoma) comparing this recMAGE-A3 + AS15 ASCI with placebo, but more exact figures cannot be provided because the data from these studies are blinded.

Most of the adverse events observed in all studies were to be anticipated and consist of local and systemic reactions (i.e., injection site reactions and constitutional symptoms such as myalgia, arthralgia, and fatigue). Almost all such events have been of Grade 1 or 2. Grade 3 symptoms were mainly general (i.e., fatigue, myalgia, and rigors/chills), except for local reactions associated with redness, pain, or swelling at the injection site; the majority of these symptoms were expected effects from using the AS15 adjuvant. Systemic Grade 3 events were rarely considered to be related to study treatment.

There were a total of 511 Serious Adverse Events (SAEs) in all clinical studies described in the Investigators Brochure (33). Of this number, only 23 events (4,5%) in four clinical studies were assessed as being possibly related to ASCI or control (placebo) treatment:

- Study 1: One Grade 3 event (injection site reaction); and one ungraded event (COPD exacerbation);
- Study 2: five Grade 4 events (fatigue, idiopathic pulmonary fibrosis, neutropenia, nonobstructive cardiomyopathy, idiopathic thrombocytopenic purpura); five Grade 3 events (pain in extremity, stress cardiomyopathy, pneumonia, Bowen's disease, eczema); four Grade 2 events (pyrexia, influenza-like illness, headache, and one unspecified event); and three Grade 1 events (pyrexia, hypersensitivity, COPD);
- Study 3: one Grade 2 event (allergic reaction);
- Study 4: one Grade 3 event (erysipelas); and two Grade 2 events (pyrexia, wound infection).

Such events have remained isolated cases, and almost all SAEs (488/511, or 95,5%) in these clinical studies were assessed by investigators as being unrelated to ASCI or control (placebo) treatment (29,33).

Biological indicators of auto-immunity have been observed infrequently. Very rare local clinical reactions (i.e. one transient episode of uveitis and one episode of localized vitiligo) and Grade 1-2 autoimmune events (i.e. hepatitis) have been recorded in three clinical studies where the recMAGE-AA3 antigen has been combined with two different immunological Adjuvant systems (i.e. CpG 7909 and AS15).

The non-related reported autoimmunity events were unexpected, as immunization against the MAGE-A3 antigen is not expected to lead to autoimmune reactions in humans. Even though a reasonable possibility of a causal association between autoimmune hepatitis and the recMAGE-A3 + AS15 ASCI has not been established, such an association cannot be excluded.

A large clinical safety database is currently available with the recMAGE-A3 protein in different formulations. These data show that these treatments have a very satisfactory safety profile, as most of the adverse events observed in all studies were to be anticipated and consist of low grade local and systemic reaction (i.e., injection site reactions and constitutional symptoms such as myalgia and fatigue). Almost all serious adverse events (488/511, or 95,5%) in these studies were rated by the investigators as being unrelated to the study immunization (29,33).

2.6.1 Rationale

Currently, the standard treatment for localized muscle invasive bladder cancer is radical cystectomy.

- **Many patients with muscle invasive bladder cancer will relapse after cystectomy**

The 10-year disease-specific and overall survival of patients with organ confined (defined as < pT3a) is 72.9% and 49.1%, rapidly decreasing to 33.3% and 22.8% for non-organ confined disease (13). There is thus a clear medical need for an additional anti-tumoral treatment in this population.

- **There is not enough evidence in favour of the routine use of adjuvant chemotherapy**

From scientific evidence so far available, it is unclear whether immediate adjuvant chemotherapy or chemotherapy at the time of relapse is superior or if the two approaches are equivalent with respect to the end-point overall survival. There is no additional effective treatment for the patient

population at high risk of relapse receiving adjuvant chemotherapy and this represents a significant unmet medical need (51).

- **MAGE-A3 is a factor of poor prognosis**

The interest in recMAGE-A3 ASCI treatment is further reinforced by the possible link between MAGE-A3 expression and shorter survival (34)

- **MAGE-A3 is tumor-specific, recMAGE-A3 + AS15 ASCI highly tolerated and shows promising Phase II results**

Taking into account the tumor-specificity of MAGE-A3, the high tolerability of recMAGE-A3 + AS15 and the promising results from the Phase II clinical trials in melanoma and lungcancer, EAU RF proposes to initiate a randomized, placebo-controlled clinical Phase II trial with recombinant MAGE-A3 (recMAGE-A3) combined with the AS15 adjuvant in patients with muscle invasive bladder cancer with MAGE-A3 expression after cystectomy.

This trial will assess whether adjuvant treatment with recMAGE-A3 + AS 15 ASCI after cystectomy is safe and effective and improves outcome of MAGE-A3 positive patients after cystectomy.

2.6.2 Rationale Protocol Amendment 4.0

GlaxoSmithKline Biologicals recently reported the negative outcome of the MAGE3-AS15-NSC-003 (ADJ) (109493 - EudraCT number: 2007-001283-73) MAGRIT study (A double-blind, randomized, placebo-controlled Phase III study to assess the efficacy of the recMAGE-A3 + AS15 Antigen-Specific Cancer Immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive Non-Small Cell Lung Cancer (53). The analysis of MAGRIT confirmed 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 Antigen-Specific Cancer Immunotherapeutic (ASCI) in NSCLC that could be corrected in ongoing studies, including the MAGNOLIA study.

Furthermore, a comprehensive review of the Phase III results, together with all other available clinical and laboratory data with various recombinant proteins tested in different diseases and settings now suggests that the anticancer activity of this technology may well be limited to a

subgroup of Stage III melanoma patients with a specific predictive gene signature (52). A few patients in early metastatic melanoma also appear to benefit from this type of treatment.

Consequently, GSK Biologicals has decided to stop further development of recMAGE-A3 + AS15 as a standalone treatment for cancer patients. This decision is not motivated by any safety concern as confirmed by all Independent Data Monitoring Comittees (IDMCs) overlooking the ASCI trials.

This decision implies that MAGE-A3 +AS15 CI will not be available for future treatment of bladder cancer patients which warrants a substantial amendment (4.0) of the MAGNOLIA study.

As of 23 September 2014, the recruitment was put on hold (sites were still allowed until 3 February 2015 to randomize patients that signed the informed consent or were in the screening process). As of Protocol Amendment 4.0, the recruitment will be stopped and the study population will be unblinded.

For patients randomized to the placebo group, no further protocol visits will be performed except for the concluding visit and no further doses will be administered. As it cannot be excluded that one or more patients may benefit from this treatment on an individual basis, patients receiving active treatment (patients not on placebo, and patients that have not already completed or interrupted their MAGE-A3 ASCI treatment) will be offered the option to continue the administration of the study treatment until the last dose is administered or until recurrence, whichever comes first, or until the patient or the investigator decides to stop the study treatment. Therefore, the study will continue only with patients from the active treatment group who will decide to stay in the study. Treatment will not be possible anymore after 30 November 2016.

During the treatment period, safety monitoring will continue as initially foreseen during the treatment period. In the best interest of the patient, additional biological samples for protocol research purposes (evaluation of the immune response, translational research) will not be taken nor collected.

The primary and secondary objectives will not be assessed as planned. All clinical data collected in the study will be analysed descriptively. By default, for each biological sample already collected in the scope of this study and not tested yet, testing will only be done if a scientific rationale remains relevant despite the premature termination of the study. In that case, testing will be in compliance with the protocol and ICF signed by the patient. The immune response will not be evaluated anymore as the immune response to IP administration was confirmed in other ASCI trials, but did not show any correlation with the potential efficacy.

3. STUDY OBJECTIVES & END POINTS

3.1 Primary Objective

The primary objective of this Phase II study is to evaluate the clinical efficacy in terms of Disease Free Survival of recMAGE-A3 + AS15 ASCI versus placebo in the overall population.

3.2 Secondary Objectives

Secondary objectives are:

- To evaluate overall survival in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy
- To evaluate Disease-free (DFS) in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy
- To evaluate Disease-free specific survival (DFSS) in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy
- To evaluate Distant metastasis-free survival (DMFS) in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy
- To evaluate the safety of recMAGE-A3 + AS15 ASCI in the overall study population, in the subpopulations with and without use of neo-adjuvant chemotherapy and in the subpopulations with and without use of adjuvant chemotherapy
- .
- Translational research:
  a) To identify a gene signature predictive to recMAGE-A3+AS15 ASCI in MIBC.
  b) To evaluate on exploratory basis a possible correlation between gene expression profile of the primary tumor and clinical efficacy of recMAGE-A3 + AS15 ASCI compared to placebo in terms of:
     - Disease-free Survival (DFS)
     - Overall survival

c) To evaluate expression of genes in a previously identified gene signature and evaluate their correlation with clinical efficacy of recMAGE-A3 + AS15 ASCI compared to placebo in terms of:
    - Disease-free Survival (DFS).
    - Overall survival.
    - Disease-free specific survival (DFSS)
    - Distant metastasis-free survival (DMFS).

d) To characterize of the tumor microenvironment and lymphocyte infiltration in the primary tumor and its recurrence lesions

As of Protocol Amendment 4.0 primary and secondary objectives of the MAGNOLIA study will not be assessed as planned. All clinical and safety data collected in the study will be analysed descriptively. Additional biological samples for protocol research purposes (evaluation of the immune response, translational research) will not be taken nor collected. By default, for each biological sample already collected in the scope of this study and not tested yet, testing will only be done if a scientific rationale remains relevant despite the premature termination of the study. In that case, testing will be done in compliance with the protocol and ICF signed by the patient. The immune response will not be evaluated anymore as the immune response to IP administration was confirmed in other ASCI trials, but did not show any correlation with the potential efficacy. See for rationale Section 2.6.2.

3.3 Primary Endpoint

Disease-free Survival (DFS):

- Defined as the time from randomization to either the date of first recurrence of the disease or the date of death (whatever the cause), whichever occurs first.
- Types of recurrence to be considered as an event include loco-regional and distant metastases.
- In addition, any death occurring without prior documentation of tumor recurrence will be considered as an event (and will not be censored in the statistical analysis) as this approach is less prone to introduce bias.

- If no event has occurred by the time of the analysis, then the time to event will be censored at the date of the last assessment of the patient in question.
- Any new primary cancer at another site, including transitional carcinoma of the upper urinary tract, will not be considered as recurrence and should be reported as a SAE.

As of Protocol Amendment 4.0, the primary endpoint will not be analysed as planned. All clinical data collected in the study will be analysed descriptively. See for rationale Section 2.6.2.

3.4 Secondary Endpoints

Overall Survival.

- Defined as the interval from randomization to the date of death, irrespective of the cause of death; patients still alive will be censored at the date of the last assessment.

Disease-free specific survival (DFSS).

- Defined as the interval from randomization to the date of first recurrence of disease or date of death due to bladder carcinoma, whichever occurs first. Patients without recurrence or death are censored at the date of last assessment. Patients without recurrence who die from another cause are censored at the date of death.

Distant metastasis-free survival (DMFS).

- Defined as the interval from randomization to the date of first distant metastasis or date of death, whichever occurs first. Patients alive and without distant metastasis are censored at the date of last assessment.

Adverse events.

- Occurrence of adverse events, including potential immune-mediated diseases

Serious adverse events.

- Occurrence of serious adverse events

To evaluate the efficacy of the recMAGE-A3 + AS15 ASCI compared to placebo in terms of DFS separately in the population presenting the predictive gene signature, and in the population of patients who do not present this signature

To evaluate the efficacy of the recMAGE-A3 + AS15 ASCI compared to placebo in terms of overall survival in the overall study population, in the population of patients presenting the predictive gene signature and in the population of patients who do not present this signature;

To evaluate the safety in the overall study population;

To evaluate Gene expression profiles of the primary tumor by microarray.

As of Protocol Amendment 4.0, the secondary endpoints will not be analysed as planned. All clinical data collected in the study will be analysed descriptively. See for rationale Section 2.6.2.

4. DESIGN OF THE STUDY

This is a multicentre, prospective, randomized, placebo-controlled, parallel group, double-blind, trial to compare the efficacy and safety of recMAGE-A3 + AS15 ASCI intramuscular injections with Placebo intramuscular injections.
The target was to enroll 273 patients to be randomly assigned to 2 treatment schedules in a 2:1 ratio, 2 patients randomized for recMAGE-A3 + AS15 ASCI versus 1 patient randomized for placebo, either directly after recovery from surgery, or after recovery from adjuvant chemotherapy. Ten European countries are participating with a total of 50 sites. Sites contributed 0-28 screened patients in the recruitment period of 40 months. Enrolment in this study was competitive.

As of 23 September 2014, the recruitment was put on hold (sites were still allowed until 3 February 2015 to randomize patients that signed the informed consent or were in the screening process).
As of Protocol Amendment 4.0, the recruitment will be stopped and the study will be unblinded (see for rationale Section 2.6.2).
For patients randomized to the placebo group, no further protocol visits will be performed except for the concluding visit and no further doses will be administered. The study will continue only with patients from the active treatment group who wll decide to stay in the study. Patients receiving active treatment (patients not on placebo, and patients that have not already completed or interrupted their MAGE-A3 ASCI treatment) will be offered the option to continue the administration of the study treatment until the last dose is administered or until recurrence, whichever comes first, or until the patient or the investigator decides to stop the study treatment. The duration of the treatment period will not exceed 108 weeks for any patient, comprising the entire period of treatment administration and excluding the concluding visit (Visit 14), provided there are no postponements. The concluding visit will take place at least 30 days after the patient's

last dose of study product. Treatment will not be possible anymore after 30 November 2016. The 2,5-year active follow-up period planned in the initial study protocol has been cancelled.
The total duration of the study will be 7 years. Data collection will be organised by Remote Date Entry on Electronic Case Report Forms (eCRF).

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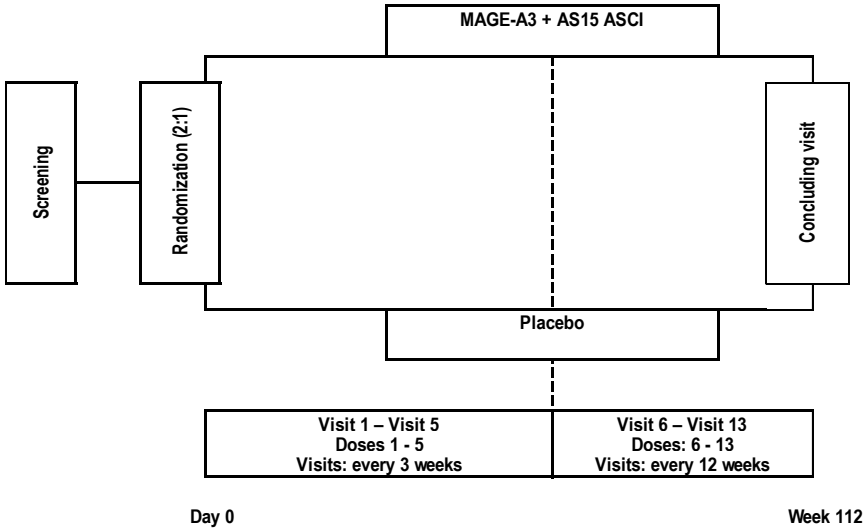
4.1 Study Treatment Administration Scheme

The treatment scheme for recMAGE-A3 + AS15 ASCI administration or placebo, consists of 5 doses of the recMAGE-A3 + AS15 ASCI or placebo administered at 3-week intervals (visit 1 to visit 5) followed by 8 recMAGE-A3 + AS15 ASCI doses or placebo administered at 3-month intervals (Visit 6 to Visit 13) for a total maximum duration of study treatment administration of 27 months. recMAGE-A3 + AS15 ASCI or placebo is given as an intramuscular injection (more info in Section 6 Investigational Products and Administration).



- Note that as of Protocol Amendment 4.0, the recruitment will be stopped and the study will be unblinded. For patients randomized to the placebo group, no further protocol visits are to be performed except for the concluding visit and no further doses are to be administered. Please see Section 2.6.2. for the rationale.

5. PATIENT SELECTION CRITERIA

5.1 Study Population

Initially, a total of 273 patients meeting all the inclusion criteria and none of the exclusion criteria, was planned to be randomized a few days before first study treatment administration with an expected average of 40% of the patients to be MAGE-A3 positive. We anticipated that we needed to screen 4 or 5 patients for each eligible patient who could be randomized. Hence, we expected to need approximately 1229 patients (range 1092 – 1365 patients) who underwent radical cystectomy and were willing to participate to end up with 273 patients who are MAGE-A3 positive and eligible to enter the study. At the time of putting the recruitment on hold (as of 23 September, 2014) 83 patients out of the 273 initially foreseen were randomized at 29 sites. Patients that already signed the screening ICF can still be randomized if eligible.

5.2 Inclusion Criteria

To be eligible for this study, patients needed to meet all of the following inclusion criteria:

1. Aged greater than or equal to 18 years at the time ICF is signed, either sex.
2. Histologically confirmed (after cystectomy or if needed transurethral resection) urothelial carcinoma of the bladder which is MAGE-A3 positive.
3. Written informed consent for tissue sampling, the mandatory analyses and for the complete study has been obtained prior to the performance of any other protocol-specific procedure.
4. TNM classification at pathological examination of surgically removed specimen: Stage $T_{2,3}$ N_0 or N_1 or N_2 and M_0 disease or Stage T_4 N_0 M_0 disease. (TNM classification see Appendix 3)
5. The patient is free of residual disease and free of metastasis, as confirmed by a negative baseline Computer Tomogram (CT scan) or Magnetic Resonance Imaging (MRI) of the pelvis, abdomen and chest no more than 13 weeks prior to randomization. Other examinations should be performed as clinically indicated.
6. Patient is fully recovered from surgery within 13 weeks following cystectomy. For patients who receive adjuvant chemotherapy, the patient is fully recovered within 3-6 weeks following chemotherapy.
7. The patient must have adequate bone-marrow reserve, defined as an absolute neutrophil count $\geq 1.0 \times 10^9$/L, and a platelet count $\geq 75 \times 10^9$/L, adequate renal function, defined as a

serum creatinine ≤ 1.5 times the Upper Limit of Normal (ULN), and adequate hepatic function, defined as a Total bilirubin ≤ 1.5 times the ULN, and a Alanine transaminase (ALAT) and Aspartate Transaminase (ASAT) ≤ 2.5 times the ULN as assessed by standard laboratory criteria.

8. World Health Organization (WHO) performance status 0 – 1 at the time of randomization. (Appendix 2)
9. If the patient is female, she must be of non-childbearing potential, i.e. have a current tubal ligation, hysterectomy, ovariectomy or be post menopausal, or if she is of childbearing potential, she must practice adequate contraception (definition see Section 7.3.1) for 30 days prior to administration of study treatment, have a negative pregnancy test and continue such precautions during all study treatment period and for 2 months after completion of the injection series.
10. The patient should be affiliated to health insurance or benefit of such an insurance

5.3 Exclusion Criteria

Patients with <u>any of the following exclusion criteria</u> were NOT eligible for the study:

1. The patient has previous or concomitant malignancies at other sites except effectively treated non-melanoma skin cancer, cervical carcinoma in situ, incidental localised prostatic carcinoma or effectively treated malignancy that has been in remission for over 5 years.
2. The patient has received any anti cancer systemic treatment, including immunotherapy (local intravesical BCG is allowed), chemotherapy, except:
   * For the treatment of previous malignancies as allowed by the protocol (i.e., non-melanoma skin cancer, cervical carcinoma in situ, incidental localised prostatic carcinoma or effectively treated malignancy that has been in remission for over 5 years).
   * For the treatment with neo-adjuvant chemotherapy for their muscle invasive bladder cancer
   * For the treatment with adjuvant cisplatinum-based chemotherapy for their muscle invasive bladder cancer
3. The patient has received radiotherapy of the abdominal or pelvic region, within 6 months prior to randomization.
4. Women who are pregnant or breast feeding.

5. The patient has a known infection with human immunodeficiency virus (HIV) or chronic hepatitis B or C.
6. The patient has a history of allergic disease or reactions likely to be exacerbated by any component of the study investigational product.
7. The patient has any confirmed or suspected immunosuppressive or immunodeficient condition or potential immune-mediated diseases as specified in Table 8. Patients with vitiligo are not excluded to participate in the trial.
8. Patient has received a major organ allograft.
9. The patient requires concomitant treatment with systemic corticosteroids, or any other immunosuppressive agents. Note: the use of prednisone, or equivalent, < 0,125 mg/kg/day (absolute maximum 10 mg/day), or inhaled corticosteroids or topical steroids is permitted.
10. The patient has received any investigational or non-registered medicinal product other than the study medication within the 30 days preceding the first dose of study medication, or plans to receive such a drug during the study.
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 other concurrent severe medical problems, unrelated to the malignancy, that would significantly limit full compliance with the study or expose the patient to unacceptable risk. For example, but not limited to: uncontrolled congestive heart failure or uncontrolled hypertension, unstable heart disease (coronary heart disease or myocardial infarction), uncontrolled arrhythmia or patients taking anticoagulant treatment or having a coagulation disorder.
13. The patient uses alternative treatments eg. plantextracts.
14. Adults under legal supervision

6. INVESTIGATIONAL PRODUCTS AND ADMINISTRATION

6.1 Treatment assignment and randomization

6.1.1 Patient identification

Patient numbers were assigned sequentially to patients as soon as they signed the first informed consent form and according to the range of patient numbers allocated to each study centre.

6.1.2 Randomization and treatment allocation

As of 23 September 2014, the recruitment was put on hold (sites were still allowed until 3 February 2015 to randomize patients that signed the informed consent or were in the screening process). As of Protocol Amendment 4.0, the recruitment will be stopped and the study will be unblinded. For patients randomized to the placebo group, no further protocol visits will be performed except for the concluding visit and no further doses will be administered. Patients receiving active treatment (i.e. those patients not on placebo, and patients that have not already completed or interrupted their MAGE-A3 ASCI treatment) will be offered the option to continue the administration of the study treatment until the last dose is administered or until recurrence, whichever comes first, or until the patient or the investigator decides to stop the study treatment. Therefore, as of Protocol Amendment 4.0, the study will continue only with patients from the active treatment group who will decide to stay in the study. Treatment will not be possible anymore after November 2016. For the rationale see Section 2.6.2.

The aim is to start study treatment as soon as the patient is recovered from surgery or, for patients who receive adjuvant chemotherapy following surgery, the patient is recovered from chemotherapy. Randomization occured maximally 13 weeks after cystectomy for patients not scheduled to receive adjuvant chemotherapy. For patients receiving adjuvant chemotherapy, the first study treatment administration must be given 3 - 6 weeks after the last day of chemotherapy administration and no more than 36 weeks after surgery.
Treatment allocation at the investigator site will be performed using a central system on Internet, initially used for randomization (SBIR; available 24H/day, 7D/week) which accounted for the following stratification factor: T category, Neo-adjuvant Chemotherapy vs. Adjuvant Chemotherapy vs no chemotherapy, and minimization factors the number (1, 2 vs. 3, 4) of cycles received (if peri-operative chemotherapy has been administered), N category, gender and centre. This randomization took place maximum 7 days before first study treatment administration at Visit 1.

After having obtained informed consent for MAGE-A3 expression screening and gene profiling and separate informed consent for the complete study and having checked that the patient was eligible, the investigator or his/her delegate accessed the randomization system via the Internet. The patient's identification number, and stratification and minimization factors: T category, Neo-adjuvant Chemotherapy vs. Adjuvant Chemotherapy vs. no chemotherapy, the number of peri-operative chemotherapy cycles received , N category, gender and centre were entered. This central system, initially used for randomization, will now determine the treatment allocated to the patient and will provide the treatment box number to be used for the immunization of this patient.
For further information on the randomization system and attribution of treatment box numbers, refer to the SBIR User Guide
The actual treatment number used for each study treatment administration of the patient must be recorded by the investigator in the eCRF.

6.2 Study Treatment

All candidate ASCI and placebo to be used in this study have been developed and manufactured by GSK Biologicals.
The Quality Control Standards and Requirements for this candidate product are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis, etc.) and the required approvals have been obtained.
The study product(s) are labeled and packed according to applicable regulatory requirements.
Treatment will be limited to 30 November 2016, as the lots of the IMP (Investigational Medical Product) produced for this trial, are expected to expire.

6.2.1 recMAGE-A3 + AS15 antigen-specific cancer immunotherapeutic

In the study, the recMAGE-A3 + AS 15 ASCI will be administered by using a sterile two vial set comprising:

- One vial with the lyophilized preparation containing 300 µg recMAGE-A3 antigen plus 420 µg of CpG7909 (a part of the adjuvant system AS15),
- One vial with liquid adjuvant diluent AS01B (containing liposomes), along with 50 µg of MPL combined with 50 µg of QS21 in phosphate buffered saline), making up the remainder of the adjuvant system AS15.

The final recMAGE-A3 + AS15 ASCI for administration is obtained by reconstitution of the lyophilized preparation with the adjuvant diluent. A recMAGE-A3 + AS15 ASCI dose consists of 0.5 ml.

6.2.2. Placebo

The placebo will consist of sucrose reconstituted with a 1/500 dilution of SB62 oil-inwater emulsion (pH = 6.8) in a total volume of 0.5 ml. It will be administered by using a sterile two-vial set comprising:

- One vial with the lyophilized sucrose preparation,
- One vial with the diluted oil-in-water emulsion diluent.

As of Protocol Amendment 4.0, the recruitment will be stopped and the study will be unblinded. For patients randomized to the placebo group, no further protocol visits are to be performed except for the concluding visit and no further doses are to be administered. Please see Section 2.6.2 for the rationale.

6.3 Dosage and Administration of Study Product

6.3.1 General considerations

A standard dose of recMAGE-A3 (300 μg) + AS15, or of placebo, will be used, irrespective of the patient's body weight or area (Table 1). The needles used for study treatment administration should be suitable for intramuscular injection.

The 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 investigator or designate will then withdraw the reconstituted AS 15 ASCI, change the needle, and 0.5 ml will be injected slowly (approximately 30 seconds) intramuscularly in the deltoid or lateral regions of the thighs, alternately on the right and left sides.

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

When reconstituted, the recMAGE-A3 + AS15 ASCI or the placebo can be kept between +4°C and +25°C for a maximum of 4 hours.

Table 1: Dosage and Administration

Treatment	Dose	Administration	
		Timing	Route and site
ASCI Arm			
recMAGE-A3 + AS15 ASCI	0.5 ml corresponding to 300 μg of recMAGE-A3 antigen and 420 μg CpG reconstituted in AS01B	Every 3 weeks from Visit 1 to Visit 5 Every 12 weeks from Visit 6 to Visit 13	IM Deltoid or lateral region of the Thigh preferably alternating on right and left side
Placebo Arm*			
placebo	0.5 ml of sucrose reconstituted in a diluted oil-in-water emulsion		

* Note that as of Protocol Amendment 4.0, the recruitment will be stopped and the study will be unblinded. For patients randomized to the placebo group, no further protocol visits are to be performed except for the concluding visit and no further doses are to be administered. Please see Section 2.6.2 for the rationale.

Criteria for permanent stopping or postponement of study treatment are described in Section 6.3.2.

6.3.2 Contra-indications to subsequent study treatment administration

The criteria in the following subsections should be checked at each visit subsequent to the first visit. If any of these events occur during the study, this will require appropriate action, i.e., interruption of the treatment with postponement of the next study treatment administration (Section 6.3.2.1) or permanent stopping of study treatment (see Section 6.3.2.2).

6.3.2.1 Criteria for postponement of study treatment

If any one of the following events occurs at the time scheduled for administration of the study treatment, the patient may be treated at a later date (i.e., the entire program of study visits and immunizations is interrupted), within the time window specified below, or withdrawn at the discretion of the investigator. The patient must be followed until resolution of the event, as with any AE.

- Acute disease at the time of administration. (Acute disease is defined as the presence of a moderate or severe illness with or without fever or any grade ≥2 Common Terminology Criteria Adverse Event (CTCAE Version 4) possibly related to the study treatment. Study treatment can be administered to persons with a minor illness such as diarrhea or mild upper respiratory infection.)
- Fever, defined as an oral, axillary or tympanic temperature of 38 °C or above.
- Need for influenza vaccine in the framework of imposed influenza vaccination programmes. The study treatment should be given as close in time as possible to the time scheduled in the original administration schedule. A seven days interval should be allowed between administration of such vaccine and the study treatment (see also Section 6.6.3 on time window for prophylactic vaccination).

- Need for administration of immunoglobulins and/or any blood products. The study treatment should be given as close in time as possible to the time scheduled in the original administration schedule. A seven days interval should be allowed between the administration of immunoglobulins and/or blood products and the administration of the study treatment (see also Section 6.6.3).
- Any other medical reason that would expose the patient to an unacceptable risk, at the investigator's discretion.

In case of postponement of study treatment administration for any of the above reasons, the following rules have to be followed:

- From visit 2 to Visit 5 (study treatment administration no. 2 to 5), the maximum delay for postponement of study treatment administration is 3 weeks.
- From Visit 6 to Visit 13 (study treatment administration no. 6 to 13), the maximum delay for postponement of study treatment administration is 12 weeks.

When a study treatment administration has to be postponed, a visit to administer the missed treatment should be planned as soon as possible to catch up the originally planned schedule.

If the administration occurs within the above stated delay, the treatment dose to administer corresponds to the next one in the initial order of administration. Example: if a patient has already received doses 1 and 2 of study treatment but the administration of dose 3 of study treatment has to be postponed, the patient will receive dose 3 if he/she comes for his next administration a maximum of 3 weeks after the originally planned date of Visit 3.
If the administration does not occur within the above stated delay, the missed dose will not be administered. At the time the patient reintegrates the study, he/she will receive the dose as planned in the initial schedule of administration. Example: if a patient has already received doses 1 and 2 of study treatment but the administration of dose 3 of study treatment has to be postponed for more than 3 weeks, the patient will receive dose 4 if he/she comes for his next administration (refer to Table 2).

The next doses will be planned at a time allowing:

- A minimum of 14 days between 2 treatment administrations,
- To keep up with the schedule as based on the date of first study treatment administration.

Table 2: Example of postponement of study treatment dose for a maximum of 3 weeks (from Visit 2 to Visit 5)[a]

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

Administration postponed for 2 weeks

2 weeks delay for next doses

a. Also applies for postponement up to ***12 weeks*** from Visit 6 to visit 13.

Table 3: Example of postponement of study treatment dose for more than 3 weeks (from Visit 2 to Visit 5)[a]

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

Administration postponed for 4 weeks

2 week delay for next dose

a. Also applies for postponement for more than ***12 weeks*** from Visit 6 to Visit 13.

6.3.2.2 Criteria for permanent stopping of study treatment

If any of the following criteria become applicable **during the study**, the patient will be required to discontinue the study treatment.

1. Evidence of disease relapse.
2. Treatment with one of the following:
   - Investigational or non-registered product other than the study recMAGE-A3 + AS15 ASCI.
   - Other anticancer treatments, including but not limited to chemotherapeutic or immunomodulating agents and radiotherapy.
3. Anaphylactic reaction following the administration of study treatment.

4. Any intolerable adverse event, **at the investigator's discretion**, such as study treatment related CTC ≥ Grade 3 AEs or SAEs.
5. Clinical signs or symptoms indicative of any autoimmune disorder (see Section 8.3); in such cases, appropriate clinical and laboratory testing will be performed to identify and characterize that disorder.
6. Appearance of any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection, or any medical condition requiring the use of any immunosuppressive agent or the use of systemic corticosteroids prescribed for chronic use (more than 7 consecutive days). Note: The use of prednisone, or equivalent, of < 0.125 mg/kg/day (absolute maximum 10 mg/day), or inhaled corticosteroids or topical steroids is permitted.
7. Inability of the patient to complete the study evaluations/visits because of unforeseen circumstances.
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. The patient and/or investigator requests the withdrawal from the study treatment motivated or not by the Study Protocol Amendment 4.0.
10. For female patients, pregnancy or decision to become pregnant.

Procedures to be performed in any of these situations are described in Section 9.2.1.

6.4 Method of Blinding

Initially the study was double-blind. As of Protocol Amendment 4.0, the recruitment will be stopped and the study will be unblinded.

6.5 Storage and Handling of Study Products

All study products to be administered to patients must be stored and handled during the course of the study as described below:

- Storage areas must be dry and clean
- Refrigerator / freezer needs to provide adequate storage conditions and needs to function properly, i.e. maintenance of T° within the T° conditions defined for the IMP
- Storage temperature must be maintained, checked and monitored
- Sufficient storage space should be foreseen for all study products provided during the course of the study
- The storage space to be used for the study products should be clearly delimited and physically separated from all other samples/items contained in the storage facility

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

Study products must be stored at the defined temperature range (i.e. +2 to +8°C).

When reconstituted, the recMAGE-A3 + AS15 ASCI can be kept at a temperature between 4°C and 25°C for a maximum of 4 hours.

For local storage between 2-8°C, a temperature monitoring system should be used to monitor and record daily temperature. The temperature should be registered on a temperature log sheet at least once per working day, preferably at the same time of the day (e.g., at the beginning of the day).

It is permitted to monitor the storage temperature using a locally available temperature continuous recording device, provided it can read the daily actual and min/max temperatures.

In case of temperature deviation Sponsor should be contacted whether affected products may still be used or not.

Please refer to the Pharmacy manual for more details on storage & handling of the study product(s).

6.5.1 Replacement of unusable study product doses

Additional study product 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 active treatment number to be used for the immunization of that patient.

6.6 Concomitant medication/vaccination and non-drug therapies

6.6.1 Permitted medication

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 taken and treatment received by the patient.

All concomitant medication/vaccination, 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.

Similarly, concomitant medication administered for the treatment of a SAE, at any time, must be recorded on the SAE Report Form/SAE screens in the eCRF, as applicable. Refer to Section 8 for the definition of a SAE.

6.6.2 Prohibited medications and non-drug therapies

Patients may not receive concomitant treatment during the study treatment period (from the first treatment administration until the concluding visit) with any of the following:

- Investigational or non-registered product other than the study recMAGE-A3 + AS15 ASCI.
- Plant extracts or anticancer treatments, including but not exclusively, chemotherapeutic or immunomodulating agents and radiotherapy.
- Any immunosuppressive agent, or any systemic corticosteroids prescribed for chronic use (more than 7 consecutive days). The use of prednisone, or equivalent, < 0,125 mg/kg/day (absolute maximum 10 mg/day), or inhaled corticosteroids or topical steroids is permitted.
- Administration of a vaccine not foreseen by the study protocol during the period within the prohibited time as specified in Section 6.6.3.

Any of these specifically contraindicated treatments and/or medications administered at any time during the study treatment period is 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 6.3.2.2) or may determine a patient evaluability in the according-to-protocol (ATP) analysis (see Section 10.3 for definition of study populations to be evaluated) .

6.6.3 Allowed time window for concomitant vaccination, immunoglobulins and blood products

Immunization with any commercial anti-infectious vaccine may be performed during the study. However, this may not take place during the period from 7 days before any study product administration to 7 days after. Thus, if the study product is to be administered on a notional Day 0, then the vaccination may be performed on or before Day -8, and on or after Day 8.
In case of need for influenza vaccine in the framework of imposed influenza vaccination programs, the ASCI treatment may be postponed in order to administrate such prophylactic vaccine. However, a 7 day interval should be allowed between the influenza vaccine administration and the ASCI treatment.
Any commercial vaccine administered in the study treatment period is to be recorded in the eCRF .
Administration of immunoglobulins and/or any blood products during the treatment period is allowed, within the same time windows as noted above for anti-infectious vaccines.

6.6.4 Time window for recording concomitant medication/vaccination and non-drug therapies in the eCRF

All concomitant medication as described in Sections 6.6.1, 6.6.2 and 6.6.3, with the exception of vitamins and/or dietary supplements, administered at ANY time during the period starting with the administration of the first dose of study product and ending 30 days after the last dose of study product must be recorded in the eCRF.

7. STUDY ASSESSMENTS AND PROCEDURES

7.1 General Study Aspects

Attendence for study visits

It is the investigator's responsibility to ensure that the intervals between visits/contacts are strictly followed.

Patients will be required to attend for study treatment administration and assessment according to the schedule shown below (Section 7.2). The first study treatment will take place maximum 7 days after randomization. Permitted deviation from the stipulated date of each visit (due e.g. to week-ends or public holidays) will be as follows:

Screening 1: prior to or shortly after cystectomy
Screening 2:
For patients not receiving adjuvant chemotherapy max 13 weeks after cystectomy
For patients receiving adjuvant chemotherapy within 3-6 weeks after the last day of chemotherapy administration
Visit 1: max 7 days after randomization and max 36 weeks after cystectomy
Visits 2-5: ± 4 calendar days.
Visits 6-13: ± 14 calendar days.
The concluding visit will take place at least 30 days after the patient's last dose of study product.
The follow-up visits planned in the initial study protocol has been cancelled.
Any deviation greater than this will be regarded as a protocol violation.

In each case, the sponsor will decide, if appropriate in consultation with the IDMC, 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 (ATP)).

When the permitted deviations are applied or when a study treatment has to be postponed (refer to Section 6.3.2.1 for details on postponement of study treatment), the next study visit should be planned at a time allowing:

- A minimum of 14 days between 2 treatment administrations,
- To keep up with the schedule as based on the date of first study treatment administration (Visit 1).

During the treatment phase, 1 week equals 7 calendar days. During the treatment phase timings will be counted in weeks.

7.2 Outline of study procedures

Table 4 below shows the schedule for procedures and assessments in this trial.

Table 4: List of study procedures from Visit S1 to Visit 5

Study phase:	Screening			Visit 1- Visit 5				
Timing of visits			max 35 weeks post cystectomy	max 7 days post randomization				
Study visit no. Study treatment administration no. Time after first study treatment administration	**S1**	**cystectomy**	**S2** [a]	**1** 1 0	**2** 2 3 w	**3** 3 6 w	**4** 4 9 w	**5** 5 12 w
Informed consent	● [b]		● [c]					
Addendum to Informed consent				● [k]	● [k]	● [k]	● [k]	● [k]
MAGE-A3 expression analysis [d]		●						
Inclusion and exclusion criteria	○		●					
Medical history			●					
Smoking status			●					
Patient randomized			●					
Efficacy assessments								
Chest, abdomen and pelvis CT scan or MRI			●	[e]	[e]	[e]	[e]	●
Physical examination, including height and weight			●					
Physical examination				●	●	●	●	●
WHO performance status			●	●	●	●	●	●
Safety assessments								
Adverse events recorded [f]				●	●	●	●	●
Potential immune-mediated diseases recorded [g]				●	●	●	●	●
Serious adverse events recorded [f]			●	●	●	●	●	●
Laboratory assessments								
Blood samples taken for:								
Safety tests (15 ml)		e	●	e	e	e	e	●
Pregnancy test (for female patients)			●	●[i]	[i]	[i]	[i]	● [h, i]
Urine sample for MAGE-A3 expression analysis (at least 30 cc)[j]	●							
Investigational product								
Criteria for permanent stopping or postponement of study treatment				●	●	●	●	●
Recording of concomitant medication			●	●	●	●	●	●
recMAGE-A3 + AS15				●	●	●	●	●

w = weeks. For permitted deviation, refer to Section 7.1. Pts = patients.

a) Visit S2 s took place after Visit S1, once the MAGE-A3 expression information was known to the investigator, and either at maximally 13 weeks following cystectomy or within 3-6 weeks after the last day of adjuvant chemotherapy administration with a maximum of 35 weeks after cystectomy.

b) Signature of informed consent for tissue sampling/analysis including informed consent for gene profiling and some optional translational research took place before or shortly after surgical resection.

c) Signature of informed consent for all other study procedures, including optional translation analysis,has taken place as soon as results of the MAGE-A3 expression test was known, and s has taken place before any further study procedures are performed.

d) MAGE-A3 screening was performed on tissue removed during surgical or transurethral resection. For the rapidity of testing and quality of the tumor block / slides, tissue was sent as soon as possible after operation.
Only tumor tissue from patients likely to meet all inclusion criteria was sent.

e) To be repeated/performed at any time if clinically indicated.

f) Refer to Section 8.

g) Refer to Section 8.3.

h) A pregnancy test must be performed every 12 weeks for female patients of childbearing potential.

i) A pregnancy test should be performed at any time during the study if the investigator/patient suspects that pregnancy has occurred. At V1 a pregnancy test should be repeated if the test at S2 was more than 7 days ago.
j) Urine sample for MAGE-A3 expression analysis was optional and should have contained at least 30 cc and should have been performed at S1 before cystectomy and at recurrence of bladder cancer in the urinary urothelium.
k) Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 4.0. 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 eCRF at visit S2.

Note that as of Protocol Amendment 4.0. no additional blood samples (for immunological examinations) or urine samples (for MAGE-A3 expression tests) will be taken and collected.

Procedures should be performed on 1. the planned visit or 2. within 14 days prior to the planned visit. Procedures should be performed before injection, not after injection of the study treatment.

Note: ● is used to indicate a study procedure that requires documentation in the individual eCRF. ○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

Table 5: List of study procedures from Visit 6 to Visit 13 and at concluding visit

Study visit no.	6	7	8	9	10	11	12	13	14 [f] Concluding visit
Study treatment administration no.	6	7	8	9	10	11	12	13	-
Time after first study treatment administration	24 w	36 w	48 w	60 w	72 w	84 w	96 w	108 w	112 w
Addendum to informed consent	●[a]	●[a]	●[a]	●[a]	●[a]	●[a]	●[a]	●[a]	●[a]
Efficacy assessments									
Chest, abdomen and pelvis CT scan or MRI	●	●	●	●	●	●	●	●	●
Physical examination	●	●	●	●	●	●	●	●	●
WHO performance status	●	●	●	●	●	●	●	●	●
Safety assessments									
Adverse events recorded [b]	●	●	●	●	●	●	●	●	●
Potential immune-mediated diseases [b]	●	●	●	●	●	●	●	●	●
Serious adverse events recorded [b]	●	●	●	●	●	●	●	●	●
Laboratory assessments									
Blood samples taken for:									
Safety tests (15 ml)	c	c	●	c	c	c	●	c	●
Pregnancy test (for female patients)	● [d e]	● [d e]	● [d e]	● [d e]	● [d e]	● [d e]	● [d e]	● [d e]	● [d e]
Investigational product									
Criteria for permanent stopping or postponement of study treatment	●	●	●	●	●	●	●	●	
Recording of concomitant medication	●	●	●	●	●	●	●	●	●
recMAGE-A3 + AS15 or placebo administration	●	●	●	●	●	●	●	●	
Treatment conclusion									●

W=weeks. For permitted deviation, refer to Section 7.1.

a) Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 4.0. 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 eCRF at visit S2. The procedures of this visit are to be carried out in case of recurrence (refer to Section7.3.3) or early withdrawal (refer to Section 9.2). In case of recurrence an optional tissue sample (FFPE or fresh tumor tissue) can be collected
b) Refer to Section 8.
c) To be repeated/performed at any time if clinically indicated.
d) A pregnancy test must be performed every 12 weeks for female patients of childbearing potential.
e) A pregnancy test should be performed at any time during the study if the investigator/patient suspects that pregnancy has occurred.

Note that as of Protocol Amendment 4.0. no additional blood and urine samples will be taken and collected for immunological examinations (on blood samples) and MAGE-A3 expression tests (on urine samples).
Procedures should be performed on 1. the planned visit or 2. within 14 days prior to the planned visit. Procedures should be performed before injection, not after injection of the study treatment.

Please note that as of Protocol Amendment 4.0 the follow-up visits (and the study procedures during follow-up phase, listed in Table 6 of the initial study protocol) have been cancelled.

7.3 Detailed Description of Study Stages Visits

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

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

7.3.1 Screening Assessments

Two screening visits, S1 and S2 took place: S1 before or shortly after surgery or adjuvant chemotherapy following surgery and S2 either after recovery from surgery or after recovery from adjuvant chemotherapy following surgery (see also section 7.1). Signature of informed consent for urine and tissue sampling, MAGE-A3 expression analysis and gene profiling and optional translational research (e.g. Urine Samples prior to cystectomy (at S1) for analysis of MAGE A3 expression have been collected in those patients who specifically consent to this) took place at Visit S1, before surgical resection.

At S1 the inclusion and exclusion criteria were evaluated. Only patients who to the opinion of the investigator, are likely to meet all inclusion criteria and none of the exclusion criteria were included in the first step of the study.

Cystectomy
A radical cystectomy was performed according to local standard practice.
The surgical procedure was NOT a study procedure.

A FFPE block sample of at least 10 mm^3 of the primary tumor - or in case a tissue block was not possible 20-25 unstained 10 µm slides and 1 unstained 5 µm slide of the primary tumor - was retained and sent to the central lab for determination of MAGE-A3 expression. If a suitable sample was obtained at cystectomy, that tumor sample from the cystectomy was sent to the central laboratory for MAGE-A3 testing. In case the cystectomy tumor sample was not suitable (for whatever reason, including the tumor sample being too small or of too low tumor content, or having limiting presence of necrosis or scarring) a tumor tissue sample collected during the preceding TransUrethral Resection (TUR) was used to be sent to the central laboratory. If the analysis showed

that the patient was MAGE-A3 negative, then the patient was informed of this and was not enrolled in the study.
In selected sites and in patients who specifically consent to this, in addition to the FFPE tissue a fresh tissue block sample of the resected tumor was collected and sent to a contracted laboratory to perform additional tumor antigens and MAGE-A3 expression testing.
For determining the eligibility of the patient only the MAGE-A3 expression result from the FFPE tissue was used.

Chemotherapy administration (if applicable)
If the patient was scheduled to receive chemotherapy, up to 4 cycles of cisplatinum-based chemotherapy could be administered, starting within 13 weeks after surgery.
This administration was not a study procedure. However, the type and number of cycles of chemotherapy administered were recorded in the eCRF.

S2 took place after Visit S1, once the MAGE-A3 expression information was known to the investigator, and either within 13 weeks following cystectomy (for patients not receiving chemotherapy) or within 3-6 weeks following adjuvant chemotherapy (for patients receiving adjuvant chemotherapy) with a maximum of 35 weeks after cystectomy. The following procedures did not all need to be done on the same day, but none of them, except standard imaging procedures as indicated below, could be performed before the patient had signed the ICF for study participation.

The following screening assessments were performed at S2:

- Informed Consent Procedures for the whole study and for the addendum as needed for patients on active treatment willing to continue the treatment.
- Pathological Result of surgical specimen and if needed to be supplemented with the Pathological Result of the transurethral resection specimen at S2. The number of cancer positive and -negative lymphnodes surgically removed from specific lymphnode regions such as the obturator, internal, external, common iliac and presacral lymphnode regions were registered.
- MAGE-A3 expression of pathologic specimen.
- Inclusion and exclusion criteria were checked.
- Cross-sectional imaging or Magnetic Resonance Imaging (CT scan or MRI of chest, abdomen and pelvis) with or without IV contrast material (CT-Urography) to exclude

upper urinary tract tumors. IVU was also acceptable to exclude upper urinary tract tumors at baseline. (These imaging procedures were considered as standard of care.)

- Medical history.
- Smoking status. Smoking is known to be a risk factor for the development of bladder cancer. In this study we would like to assess the impact of (stopping) smoking on the development of a recurrence or progression by means of a questionnaire.
- Physical examination (including height and weight).
- WHO performance status.
- Blood sample for safety analysis.
- SAEs that were related to study procedures should have been reported from the date of signature of informed consent at S2.
- Potential Immune-Mediated Diseases (pIMD)
- Concomitant Medication.
- Pregnancy test (females of childbearing potential only). Pregnancy test results must have been negative at screening.

When considering the enrolment of females of childbearing potential the following definition was used:

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)

When all screening assessments had been performed and the patient met all the inclusion and none of the exclusion criteria, the patient was randomized via the internet (see Section 6.1.2).

7.3.2 Study Procedures

Visit 1-Visit 5

Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 4.0. 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 eCRF at visit S2.

First study treatment administration (V1) will take place maximum 7 days after randomization. V2 – V5 will take place 3 weeks ± 4 days after the day of first administration (V1).

During Visit 1 to Visit 5 the following procedures will be performed:

- Recording of concomitant medication (each visit)
- Recording of (serious) adverse events and potential Immune-Mediated Diseases (each visit)
- Routine physical examination including WHO performance status assessment (each visit)
- Cross-sectional imaging or Magnetic Resonance imaging (CT scan or MRI of chest, abdomen and pelvis) at V5
- Pregnancy test at V5

  A pregnancy test needs to be repeated at V1 in case the previous pregnancy test is more than 7 days ago. A pregnancy test will be repeated every 3 months for females of childbearing potential and should be performed at any time during the study for all women of childbearing potential if the investigator or patient suspects that pregnancy has occurred while the subject was being treated on protocol therapy.
- Blood sampling for safety at V5

Note that as of Protocol Amendment 4.0, blood sampling for immunological examination at V1 (pre-administration), V3 and V5 and urine sampling at the time of recurrence of bladder cancer in the urinary urothelium, for analysis of MAGE A3 expression (optional, if patient specifically consents to this), as mentioned in the original protocol, will not be performed anymore. Before each administration the criteria for permanent stopping or postponement of study treatment (Sections 6.3.2.1 and 6.3.2.2) will be checked. If any of these criteria is met, the patient will be withdrawn from study treatment or the administration of study treatment will be postponed.

Visit 6 – Visit 13

Signature of the ICF addendum must be the first procedure performed for patients returning for their first study visit after approval of Protocol Amendment 4.0. 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 eCRF at visit S2.

These visits will take place at 12-weekly intervals i.e. at time points 24, 36, …108 weeks after V1, with a permitted deviation of up to 14 days.

During Visit 6 to Visit 13 the following procedures will be performed:

- Recording of concomitant medication (each visit)
- Recording of (serious) adverse events and potential Immune-Mediated Diseases (each visit)
- Routine physical examination including WHO performance status assessment (each visit)
- Cross-sectional imaging or Magnetic Resonance Imaging (CT scan or MRI of chest, abdomen and pelvis) (each visit)
- Pregnancy test (each visit)

  A pregnancy test will be repeated every 3 months for females of childbearing potential and should be performed at any time during the study for all women of childbearing potential if the investigator or patient suspects that pregnancy has occurred while the subject was being treated on protocol therapy.
- Blood sampling for safety at V8, and V12

Note that as of Protocol Amendment 4.0, Smoking status (V6, V8, V10, V12), blood sampling for immunological examination (at V7, V8 and V10) and urine sampling (at the time of recurrence of bladder cancer in the urinary urothelium, for analysis of MAGE A3 expression, optional, if patient specifically consents to this), as mentioned in the original protocol, will not be performed anymore.

Before each administration the criteria for permanent stopping or postponement of study treatment (Sections 6.3.2.1 and 6.3.2.2) will be checked. If any of these is met, the patient will be withdrawn from study treatment or the administration of study treatment will be postponed.

Visit 14: Concluding visit

This visit will take place at least 30 days after V13, approximately 120 weeks after first administration (V1) with a permitted deviation of up to 14 days, or in case of recurrence or early withdrawal.

The following procedures will be performed:

- Recording of concomitant medication
- Recording of (serious) adverse events and potential Immune-Mediated Diseases
- Routine physical examination including WHO performance status assessment
- Cross-sectional imaging or Magnetic Resonance Imaging (CT scan or MRI of chest, abdomen and pelvis)
- Pregnancy test

  A pregnancy test will be repeated every 3 months for females of childbearing potential and should be performed at any time during the study for all women of childbearing potential if the investigator or patient suspects that pregnancy has occurred while the subject was being treated on protocol therapy.
- Blood sampling for safety

Note that as of Protocol Amendment 4.0, Smoking status and blood sampling for immunological examination (optional, if patient specifically consents to this), as mentioned in the original protocol, will not be performed anymore.

As of Protocol Amendment 4.0 the follow-up visits have been cancelled.

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7.3.3 Recurrence or progression

All types of recurrence will be included i.e. local, regional and distant metastasis as well as second primary tumor of the upper urinary tract. In addition, all deaths occurring without prior documentation of tumor recurrence will be considered as an event. Any new primary cancer will not be considered as recurrence and should be reported as SAE.

All recurrences must be documented by radiological evidence or biopsy, as determined by the investigator.

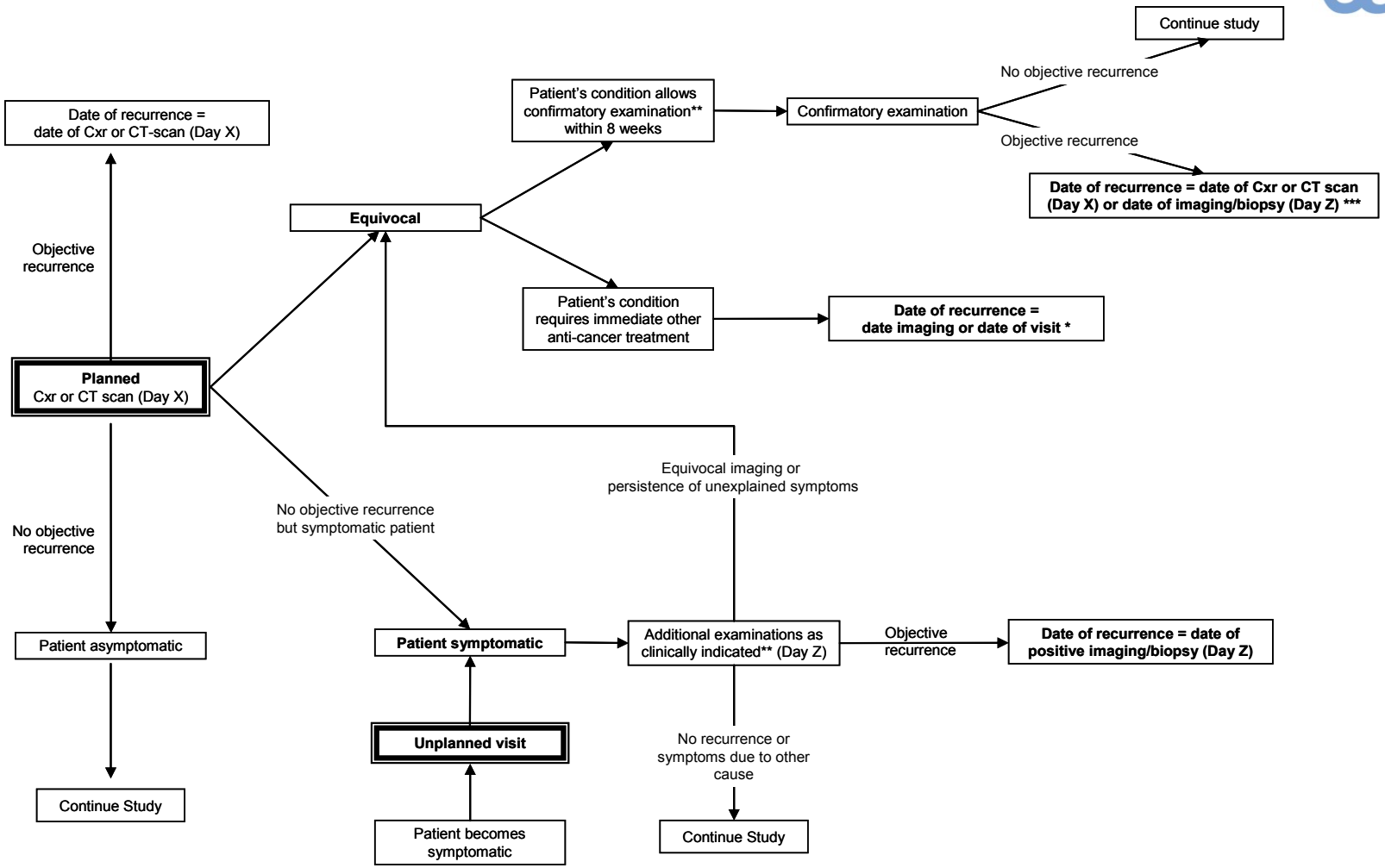
Suspicion of relapse based on physical examination findings or laboratory signs must be confirmed by radiological examination and/or biopsy.

Equivocal findings on standard radiological imaging should be confirmed by repeated examinations or cytology/histology or other imaging techniques.

In the event of unequivocal, objective recurrence (at scheduled or unscheduled visit), the investigator will determine and record the recurrence, withdraw the patient from the study treatment

and perform all procedures as defined for Visit 14 before administration of chemotherapy to treat the recurrence. The investigator must inform the Sponsor as soon as possible.

The algorithm below summarizes how the date of recurrence should be determined.



Cxr, Chest X-Ray.

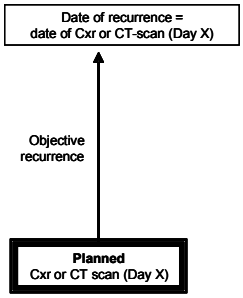
* Date of imaging if equivocal, date of unplanned visit if symptoms present without imaging evidence.

** CxR, CT scan/MRI, Bone scan, Biopsy of suspect lesion, etc.

*** Date of initial imaging (Day X) if equivocal, date of additional examinations (Day Z) if patient initially symptomatic without objective recurrence

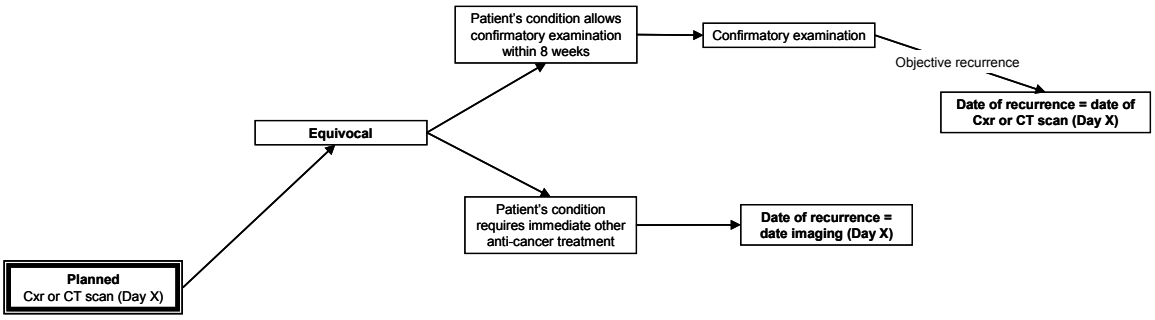
Scenario A: As soon as a planned CT scan /MRI shows objective recurrence, the patient will be considered to have had a recurrence on the day of this planned imaging (Day X).

Scenario A



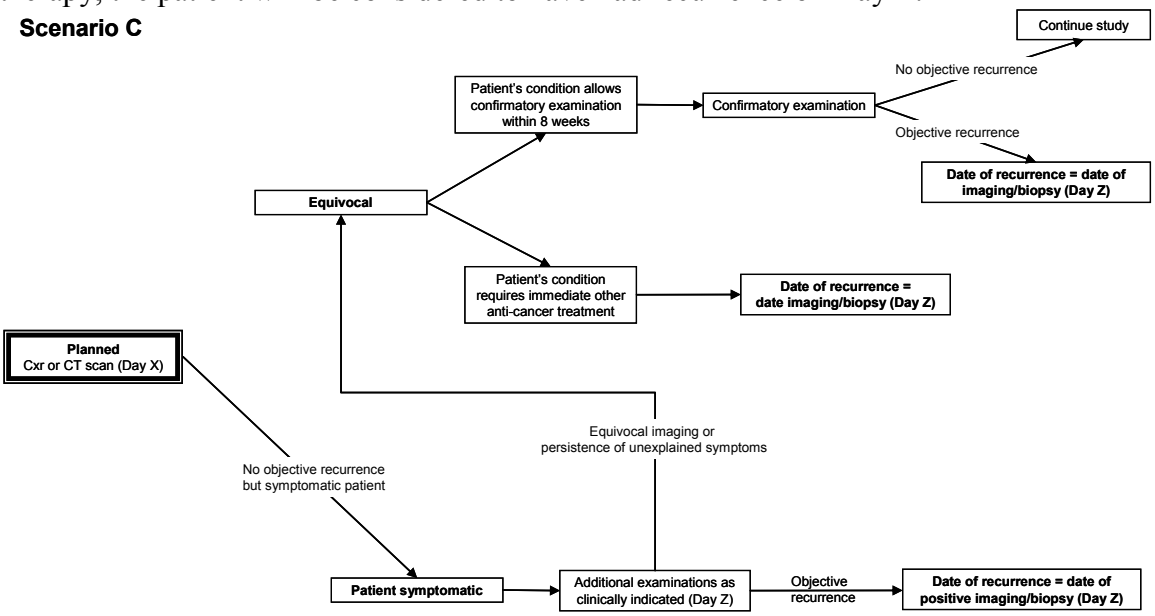
Scenario B: If a planned imaging is equivocal, a confirmatory examination (imaging or biopsy) will be planned within 8 weeks after the first equivocal imaging. If this confirmatory examination shows an objective recurrence, the patient will be considered to have had a recurrence on the day of the first equivocal imaging (Day X). On the other hand, if the patient's condition does not allow for this confirmatory examination and another anti-cancer treatment is started, the patient will be considered to have had recurrence on the day of the first equivocal examination (Day X).

Scenario B



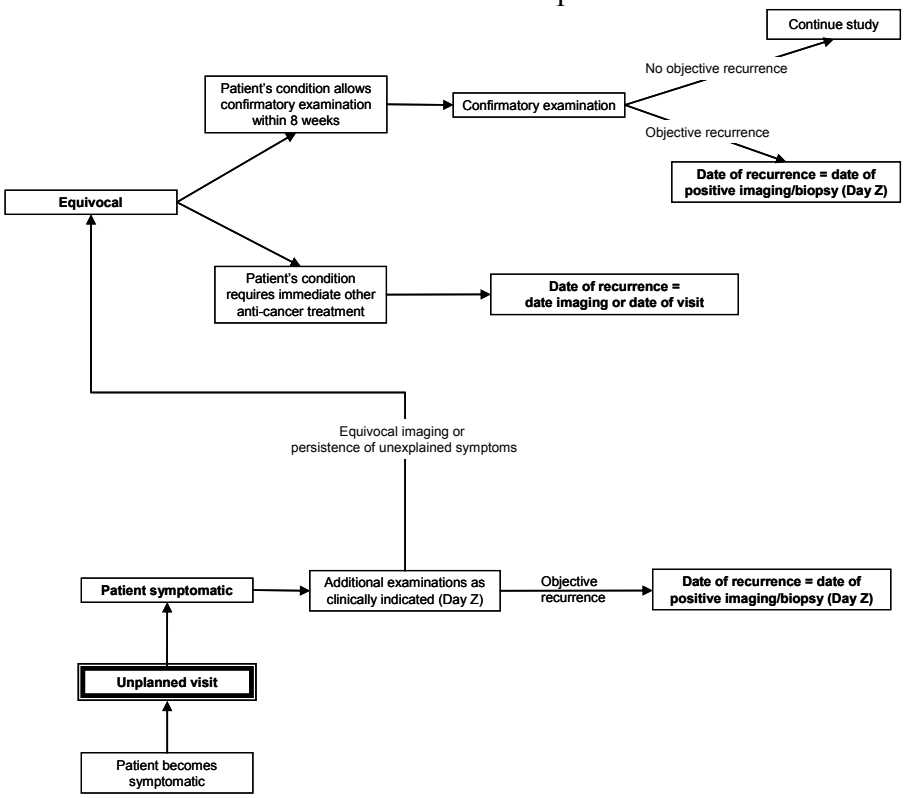
Scenario C: If a planned imaging does not show any objective recurrence but the patient is symptomatic, additional examinations will be conducted as clinically indicated (Chest X-Ray, CT scan/MRI, bone scan, biopsy, etc.). If objective recurrence is demonstrated, the date of this additional imaging or biopsy will be considered as the date of relapse (Day Z).
However, if these additional examinations are equivocal or if symptoms persist without any explanation, confirmatory examination will be performed within 8 weeks as in scenario B. If recurrence is confirmed or if the patient requires immediate treatment with another anti-cancer therapy, the patient will be considered to have had recurrence on Day Z.

Scenario C



Scenario D: If a patient comes to an unplanned visit due to symptoms, additional examinations will be conducted as clinically indicated (Chest X-Ray, CT scan/MRI, bone scan, biopsy, etc.). If objective recurrence is demonstrated, the date of this additional imaging or biopsy will be considered as the date of relapse (Day Z).
If these additional examinations are equivocal, confirmatory examination will be performed within 8 weeks as in scenario B. If recurrence is confirmed or if the patient requires immediate treatment with another anti-cancer therapy, the patient will be considered to have had recurrence on Day Z. However, if these additional examinations are negative but symptoms persist without any explanation, and the patient requires immediate treatment with another anti-cancer therapy, the patient will be considered to have had recurrence on date of unplanned visit.

Scenario D



In case of a bladder cancer recurrence a urine sample at the time of recurrence was collected and intended for analysis of MAGE A3 expression. In case a biopsy (resected tissue) of the recurrence was performed, we were interested in testing the recurrence for MAGE-A3 expression and in performing gene profiling research on the samples taken during biopsy.
The patient has signed a specific separate informed consent form to allow these tests. Instruction for sample handling and dispatch were given in the Laboratory Manual.
As of Protocol Amendment 4.0. no additional urine samples and/or biopsy tissues will be taken and collected for these tests.

7.4 Biological Sample Handling and Analysis

Table 7 summarizes all biological sample assays to be performed during this study.

As of Protocol Amendment 4.0 no more biological samples for protocol research purposes will be taken. This applies to every research topic (mandatory or optional) listed in this chapter, except for the sampling done for safety evaluation (hematology, renal function and hepatic function). 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 despite the premature termination of the study. In this case, testing will be done in compliance with the protocol and ICF signed by the patient. The immunological read-outs will not be performed anymore. Please see Section 2.6.2 for the rationale.

7.4.1. Treatment and storage of biological samples

See Laboratory manual.

Table 7: Laboratory Assays

Assay type	Test	Sample type	Laboratory	Timing
MAGE-A3 expression (mandatory at screening)*				
	MAGE-A3 expression testing	FFPE block 10mm³ (or exceptionally 20-25 slides)	GSK Bio or contracted lab	During screening and in case of recurrence (in the latter case, after patient consent has been obtained)
Laboratory assays (mandatory)				
	Hematology White blood cell count Neutrophils Platelets Lymphocytes Hemoglobin ***Renal function*** Creatinine ***Hepatic function*** Serum bilirubin Aspartate transaminase (ASAT) Alanine transaminase (ALAT) Alkaline phosphatase	Blood (15ml)	Investigator laboratory	1. During screening (Visit S2), 2. Visit 5 3. Every year post-Visit 1 until concluding visit (i.e. at Visit 8 and 12), 4. At concluding visit (Visit 14).
Translational research (mandatory)				
	Gene profiling			
	Gene profiling of primary tumor	FFPE tumor tissue	GSK Bio, Sponsor or contracted lab	After resection
Translational research (optional)				

MAGE-A3 expression testing	Urine (min 30 cc)	GSK Bio, Sponor or contracted lab	During screening (S1) and in case of recurrence in urinary urothelium
Gene profiling of recurrent tumor [b]	FFPE tumor tissue	GSK Bio, Sponsor or contracted lab	In case of recurrence
Tumor antigen and MAGE A3 expression [c]	FFPE or Fresh tumor tissue	GSK Bio, Sponsor or contracted lab	
Gene profiling of primary or recurrent tumor [c]	Fresh tumor tissue	GSK Bio, Sponsor or contracted lab	
Demethylation [c]	Urine/blood / RNALater or FFPE tumor tissue	GSK Bio, Sponsor or contracted lab	
Tumor-related DNA Biomarkers [c]	RNALater or FFPE tumor tissue/Urine	GSK Bio, Sponsor or contracted lab	
Analysis of tumor environment	RNALater or FFPE tumor tissue/Urine	GSK Bio, Sponsor or contracted lab	

a) A total of 2 x 5 ml of blood will be used for immunological read-outs
b) Including MAGE-A3 expression testing
c) No additional samples are needed for this analysis

*Note that as of Protocol Amendment 4.0 recruitment will be stopped and no more screening (and MAGE-A3 expression testing) will take place. During the treatment period, blood sampling and testing for safety monitoring will continue as initially foreseen during the treatment period, No more biological samples for immunological readouts or translational research will be collected. On the biological samples already collected, laboratory assays will only be performed if a scientific rationale remains relevant despite the premature termination of the study. The immunological read-outs will not be performed anymore. See Section 2.6.2 for the rationale.

Samples will not be labeled with information that directly identifies the patients but will be coded with a unique number for the sample.

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 product or disease under study. Therefore, all patients will be invited to give their separate agreement (tick box in the Informed Consent form) to allow the sponsor or the company supplying the trial medication and its collaborating labs to perform such additional testing.

7.4.2 MAGE-A3 expression

In order to be eligible for this study, the patients must have had a MAGE-A3-positive tumor. Therefore, the MAGE-A3 expression of the resected tumor was tested before the enrolment of the patients, i.e. during the screening period of the study.

The screening of tumors for MAGE-A3 expression was performed in a contracted laboratory located in Europe selected for its experience in the field of assays on formalin-fixed paraffin-embedded (FFPE) tissues and meeting the required quality level.

7.4.3 Safety Laboratory Assays (mandatory)

Safety laboratory tests assessing the haematological parameters as well as renal and hepatic functions will be performed at the local investigator laboratory.
These tests (detailed in Table 7) will be performed before first study treatment administration, at Visit 5 and then each year after Visit 1 (Visit 8 and Visit 12) till the end of the treatment administration phase and at Visit 14 (Table 5). Additional tests can be performed if clinically indicated and can be recorded in the eCRF.

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.

7.4.4 Immunological read-outs

7.4.4.1 Antibody response to the ASCI components

As of Protocol Amendment 4.0 no more blood samples for immunological read-outs will be taken and collected. The antibody response will not be evaluated anymore as the immune response to IP administration was confirmed in other ASCI trials, but did not show any correlation with the potential efficacy.

7.4.5 Translational research

As of Protocol Amendment 4.0 no additional biological samples for translational research will be collected. 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 despite the premature termination of the study. In this case testing will be done in compliance with the protocol and ICF signed by the patient. See for rationale Section 2.6.2.

7.4.5.1. Gene profiling research

Gene profiling research might be done for all patients enrolled in the study.

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 (35-37).
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 that, however, had no predictive correlation with clinical outcome (38, 39).
In the Phase II melanoma trial (GSK 249553/008), an attempt was made to identify from a tumor biopsy a gene signature that may predict a favorable clinical outcome for patients treated with the recMAGE-A3 + AS15 ASCI (40). In this study, the same tumor samples that were tested for MAGE-A3 expression (eligibility criteria) and so obtained before any MAGE-A3+AS15 ASCI immunisation were also tested for gene expression using Affymetrix HG-U133.Plus 2.0 gene chips covering about 47,000 transcripts. From this analysis, a specific gene signature that could segregate the patients with progressive disease and the patients with clinical activity was identified, suggesting an association between the gene signature and a favorable clinical outcome following the recMAGE-A3+AS15 ASCI treatment. A similar analysis in the Phase II NSCLC trial (GSK 249553/004) with patients treated with MAGE-A3 + AS02B ASCI, revealed that this melanoma signature might also have a predictive value in this setting.

The tumor samples in this studymight be used to identify a gene signature predictive to recMAGE-A3+AS15 ASCI in bladder cancer. In addition to attempting to identify such a signature on an exploratory basis, the incidence of previously found recMAGE-A3 +AS15 ASCI predictive gene signature and its potential predictive value in this patient population might be assessed.

Extracted RNA will be used to measure candidate predictive gene expression levels by quantitative RT-PCR or by other appropriate technologies that might interrogate the whole genome such as hybridization to a gene expression chip with a locked set of genes (e.g., *Affymetrix* or other commercial chips) or transcriptome sequencing. In case a whole genome approach is used, further gene expression profiling and validation by quantitative RT-PCR or any updated technique might be performed. Validation of gene expression at the protein level might also be performed by IHC or an appropriate technology.

This research on gene profiling might be performed on the FFPE tissue used for the mandatory MAGE-A3 expression screening (inclusion criteria for the study). If available, RNALater collected fresh tissue can be used from patients who gave explicit consent (optional testing). In addition, in the event that the investigator has performed an additional tumor biopsy or a tumor resection

because of disease progression; patients have been proposed to sign a separate Informed Consent Form to allow the tumor sample to be sent to a validated laboratory designated by GSK Bio or EAU-RF for the research on gene profiling.

7.4.5.2. Other Translational research (optional)

This section provides details on the research that may be done on the biological samples collected during this study based on sample availability and results obtained in the study. These different tests will only be performed ***if*** samples ***are available and for*** patients who voluntarily gave their consent for these specific assessments on the Informed Consent Form. Refusal to participate in this optional research will involve no penalty or loss of benefits to which the patient would otherwise be entitled. These tests will be performed at a validated laboratory designated by GSK Bio or EAU-RF.

7.4.5.2.1 Tumor antigen and MAGE –A3 expression testing

Tumor antigen-expression analysis might be carried out using same tumor samples and methodologies used for gene expression profiling (in RNA*later*® or FFPE).
The expression of tumor antigens besides MAGE-A3 (such as WT1, MAGE-C2, NY-ESO1, LAGE1, PRAME) or other related tumor antigens might be assessed at the mRNA or protein level (by qRT-PCR or immunohistochemistry respectively or any equivalent technology), using the most updated techniques at the time of the analysis.
This analysis might give a view on the prevalence of expression of these tumor antigens in bladder cancer as well as their co-expression.

If available, a fresh portion (10 mm^3) of the resected tumor collected in RNALater was sent to a contracted laboratory to perform additional MAGE-A3 expression testing. Also a urine sample taken prior to cystectomy might be analysed for MAGE-A3 expression.

7.4.5.2.2 Demethylation analysis

Gene methylation is a control mechanism that regulates gene expression in DNA. Gene methylation occurs when a methyl group is added to a cytosine nucleoside. The regulatory regions of active genes are sensitive to methylation. In normal cells, these promoter regions are regulated, as necessary, by the methylation process. In several diseases, the promoter regions can be abnormally methylated, in which case their function remains blocked and the proteins they code for are not produced. In situations where the promoter is hypo-methylated, an abnormally high level of protein can be produced. Such abnormal methylation of relevant 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 (41, 42). Analysis of methylation patterns in promoters of candidate genes that correlate with cancer aggressiveness or that predict response to the MAGE-A3 ASCI therapy could render a tool to select patients that would most benefit from this therapy**.**

Tumor cell DNA can be detected from the blood of melanoma patients even in early disease (43). This DNA can for instance originate from the primary tumor where some cells are lysed or from circulating tumor cells releasing DNA material in the blood. Likewise in bladder cancer, cancerous cells and/or their DNA can be found in the urine of patients.

It has been shown that the MAGE protein expression is re-activated in tumor cells following demethylation of the MAGE promoter region (44). The presence of MAGE-A3 expressing tumor cells could therefore be anticipated if the presence of demethylated MAGE promoter DNA is detected in the patient's blood or urine. The detection of MAGE-A3 specific demethylated DNA from these samples could lead to the development of non-invasive methods to identify the presence of MAGE-A3 expressing tumor cells. It could also potentially be used to anticipate the efficacy of the treatment if the level of the circulating DNA is decreased over time.

Practically, this approach could broaden the patient population suitable for a treatment with MAGE-A3 + AS 15 ASCI compared to the traditional methods of MAGE-A3 detection by RT-PCR on a tumor sample.

Likewise, the methylation status of regulatory regions of genes encoding other tumor antigens (such as MAGE-C2, NY-ESO1, LAGE1, PRAME and WT1) as well as genes encoding specific predictive markers and genes encoding transcriptional factors relevant for the regulation of the immune response (such as Foxp3, GATA-3, T-bet, ROR-γT) might also be analyzed in blood, urine and tumor samples.

A urine sample collected at screening might be used for the demethylation analysis. A fraction of the stored blood collected over time for immunological analysis will be used for the methylation analysis. DNA methylation might also be assessed on DNA extracted from tumor samples collected in RNA*later*® (or equivalent) or FFPE dependent on the availability of material. Therefore, no additional blood samples needed to be collected for this type of research.

7.4.5.2.3. Tumor-related DNA Biomarkers

It has been shown that changes in DNA sequence occurring in cancer cells themselves might correlate with response to a given treatment (*BRAF* in melanoma, *EGFR* in lung cancer, *KRAS* in colon cancer). (46, 47, 48, 49, 50)

This investigationwould involve the search and analysis of variant genes in the cancerous cells, which would have been shown or hypothesized to be associated with the effects of the MAGE-A3 ASCI (inflammatory or immune response genes) or that have been shown or hypothesized to play a role in cancer development, recurrence or response to other cancer treatments (*p53, RB, BRAF*, *KRAS*, *EGFR*, etc).

The analysis of genetic variability occurring in cancer cells might be performed on DNA extracted from any of the collected tumor and/or urine samples when material is available and of appropriate quality. Of note, this investigation of the tumoral cells did not require collection of additional material.

The investigation might be carried out by using hypothesis-driven approaches (candidate genes) based on PCR or alternative methodologies, such as high-throughput genotyping technologies or by using systematic approaches based on sequencing and/or whole-genome genotyping techniques that have been recently developed. The latter allow the non-biased search of genetic variations that might correlate with treatment response. In general, these analyses would aim at determining DNA sequence variations (mutations, insertion/deletion, translocations and copy number variation) that occur in the regulatory or coding region of genes.

Tumor-related analysis of samples from patients who did not receive study treatment or for whom clinical outcome is not available would be limited to establish the prevalence of specific biomarkers or to the study of the markers as prognostic factors when the follow-up information is available.

The specific type of tumor-related DNA biomarker investigation to be applied will depend on the most scientifically feasible approach available. This analysis might be done and validated using appropriate methodologies implemented in laboratories approved by EAU-RF.

7.4.5.2.4. Tumor microenvironment analysis

We intended to characterize in the primary and recurrent tumor tissue samples, the presence and characteristics of the immune infiltrate, as well as characterize the tumor microenvironment, using histopathological and/or proteomic technologies such as IHC or any other appropriate technology available.

8. 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.

8.1 Definition of an Adverse Event

AEs will be recorded according to the International Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.
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 unfavourable 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 an AE include:

- 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 they 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 study product administration.

Examples of an AE DO 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.
- 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.

Example of events to be recorded in the medical history section of the CRF/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 study product administration).

8.2 Definition of a Serious Adverse Event

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

- Results in death.
- 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.
- Requires hospitalization or prolongation of existing hospitalization.
  NB: In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) 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 fulfils 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.
  NB: Hospitalization for elective cystectomy is not an SAE.
- 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, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect in the offspring of a study patient.
- Is a Grade 4 adverse event according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 4 (Appendix 4).

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 second primary cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

8.3 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 Table 8.

However, the investigator will exercise his/her medical and scientific judgement 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.

Table 8: List of potential immune-mediated diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy), • Optic neuritis • Multiple sclerosis (including variants) • 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 diffuses systemic form and CREST syndrome and morphoea) • Systemic sclerosis Idiopathic inflammatory myopathies, including Dermatomyositis,Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis and associated conditions including Juvenile chronic arthritis, Still's disease •) • Polymyalgia rheumatica • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Ankylosing spondylitis • 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**	**Metabolic diseases**
• 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 thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease• Polyglandular autoimmune syndrome • Autoimmune hypophysitis
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 (allergic granulomatous angiitis), thromboangiitis obliterans (Buerger's disease), necrotizing vasculitis, allergic granulomatous angiitis, Henoch-Schonlein purpura, anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Behcet's	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenias • Antiphospholipid syndrome • Pernicious anemia • 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	

syndrome, leukocytoclastic vasculitis.	retinopathy) • Autoimmune myocarditis/cardiomyopathy • Sarcoidosis • Stevens-johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon

When there is enough evidence to make any of the above diagnoses, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as 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 CRF, a pIMD standard questionnaire will be available to investigators at study start.

Reporting of pIMDs

The standard time period for collecting and recording of pIMDs will begin at the first receipt of study medication and will end at the concluding visit.

Once onset of any 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, date and sign an SAE Report Form and forward it to the Sponsor WITHIN 24 HOURS after he/she becomes aware of the diagnosis. A field on the SAE Report Form allows to specify that the event is a pIMD and whether it is serious or non serious. The SAE Report Form 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 form should still be completed and forwarded to the Sponsor within 24 hours. Once additional relevant information is received, the form should be updated and forwarded to the Sponsor WITHIN 24 HOURS.

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

Fax transmission or a scanned report per e-mail are the preferred methods to forward the SAE Report Form to the Study Contact for Reporting SAEs. In absence/dysfunction of fax/e-mail equipment, the Study Contact for Reporting SAEs must be notified by telephone within 24 hours. As soon as the fax/e-mail equipment is working again, the investigator (or designate) must fax/e-mail the SAE Report Form to the Study Contact for Reporting SAEs within 24 hours.

8.4 Events or outcomes not qualifying as adverse events or serious adverse events / Progression / Recurrence of the Disease under study

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

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

However, if the progression of the underlying disease is greater than that which would normally be expected for the patient, or if the investigator considers that there was a causal relationship between treatment with recMAGE-A3 +AS15 or protocol design/procedures and the disease progression/recurrence, then this must be reported as a SAE.

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

8.5 Clinical Laboratory Parameters and other Abnormal Assessments Qualifying as Adverse Events and Serious Adverse Events

Abnormal laboratory findings (e.g., clinical chemistry, haematology, 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 8.1 or SAE, as defined in Section 8.2.

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 as well as individual laboratory values. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the

disease being studied, unless judged by the investigator as more severe than expected for the patient's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

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

8.6 Detecting and recording adverse events, serious adverse events

Time Period, Frequency, and Method of Detecting Adverse Events and Serious Adverse Events

All non-serious AEs occurring within the period beginning at the first administration of study treatment and ending 30 days after the last administration of study treatment must be recorded on the Adverse Event form in the patient's eCRF, irrespective of intensity or whether or not they are considered study treatment-related.

All SAEs must be recorded from the first administration of study treatment till the concluding visit (Visit 14, Week 112 or later) and at least 30 days after the last administration of study treatment in the event of early discontinuation of study treatment administration.

Additionally, in order to fulfil international reporting obligations, SAEs that are related to study procedures will be reported from the date of signature of informed consent. However, cystectomy before the first administration of study treatment is not considered as a study procedure. Therefore, foreseeable AEs and SAEs specifically related to the cystectomy prior to first study treatment administration should not be reported. See Section 8.9 for instructions for reporting and recording SAEs.

Table 9 summarising the periods for AE and SAE reporting during the whole study.

Study activity	Screening phase	Treatment phase			Concluding visit		
	From ICF [b] signature to Visit 1	Visit 1	Visit X (X=2 to 13)	30 days post Visit X	Visit 14		
Reporting of AEs							
Reporting of SAEs							
Reporting of potential Immune Mediated Diseases							
Reporting of SAEs related to study participation [a]							
Reporting of SAEs related to study treatment							
Reporting of Pregnancy							

a. Cystectomy is not considered as study procedure; SAEs related to this surgery should not be recorded.
b. ***ICF for study participation***

All AEs either observed by the investigator or one of his clinical collaborators or reported by the patient spontaneously or in response to a direct question will be evaluated by the investigator. AEs not previously documented in the study will be recorded in the Adverse Event form within the patient's eCRF. The nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to study treatment administration should be established. Details of any corrective treatment should be recorded on the appropriate page of the eCRF. Refer to Section 6.6. 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 treatment or since the previous visit?" N.B. The investigator should record only those AEs having occurred within the time frame defined above.

AEs already documented in the eCRF, i.e. at a previous assessment, and designated as "not recovered/not resolved" or "recovering/resolving" should be reviewed at subsequent visits, as necessary. If these have resolved, the documentation in the eCRF should be completed.

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 or designate will then record all relevant information regarding an AE/SAE in the

eCRF and on the SAE Report Forms. It is not acceptable for the investigator to send photocopies of the patient's medical records to the Sponsor in lieu of the appropriate completed AE/SAE pages in the eCRF and SAE Report Form. However, there may be instances when copies of medical records for certain cases are requested by the Sponsor. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to the Sponsor.
Paper SAE Report Forms and the facsimile (fax)/e-mail system will be the primary modes for reporting SAEs to the Sponsor during the study period. SAEs should also be reported on the SAE screens in the electronic system.
The investigator will attempt to establish a diagnosis of 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.

8.7 Evaluating Adverse Events and Serious Adverse Events

8.7.1 Assessment of intensity

Severity of AEs will be assessed according to the International Common Terminology Criteria for Adverse Events (CTCAE); version 4.0; Appendix 4.
The investigator will make an assessment of intensity 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 Adverse Event form within the patient's eCRF, SAE screens in the eCRF and SAE Report Form should be assigned according to the table given in the CTCAE (Version 4.0; Appendix 4).
An AE that is assessed as Grade 3 (severe) should not be confused with an 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 8.2.

8.7.2 Assessment of causality

The investigator is obligated to assess the relationship between the investigational product and the occurrence of each AE/SAE. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the Investigator Brochure and/or Product Information, for marketed products, 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 the Sponsor. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE to the Sponsor. 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 treatments, it may not be possible to determine the causal relationship of general AEs to the individual treatments administered. The investigator should, therefore, assess whether the AE could be causally related to study treatment administration rather than to the individual treatments.

Causality of all AEs should be assessed by the investigator using the following question: Is there a reasonable possibility that the AE may have been caused by the investigational product ?

NO : The AE is not causally related to administration of the study treatment. There are other, more likely causes and administration of the study treatment is not suspected to have contributed to the AE.

YES : There is a reasonable possibility that the treatment contributed to the AE.

Non-serious and serious AEs will be evaluated as two distinct events. If an event meets the criteria to be determined “serious” (see Section 8.2 for definition of serious adverse event), it will be examined by the investigator to the extent to be able to determine ALL contributing factors applicable to each serious adverse event.

Other possible contributors include:

- Medical history
- Other medication
- Protocol required procedure
- Other procedure not required by the protocol
- Erroneous administration
- Other cause (specify).

8.8 Follow-up of Adverse Events and Serious Adverse Events and Assessment of Outcome

After the initial AE/SAE report, the investigator is required to proactively follow each patient and provide further information to the Sponsor on the patient's condition. All AEs and SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts.

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, the event is otherwise explained, or the patient is lost to follow-up; or
- in the case of other non-serious AEs, 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 an abnormality noted for any patient must be made available to the Study Monitor.

The Sponsor may request that the investigator perform or arrange for the conduct of supplemental measurements 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, the Sponsor will be provided with a copy of any available post-mortem findings, including histopathology. New or updated information will be recorded on the paper SAE Report Form as well as on the originally completed SAE screens in eCRF. The paper SAE Report Forms should be re-sent by facsimile (fax)/e-mail system to the Sponsor within 24 hours of receipt of the follow-up information as outlined in Section 8.9.1. Refer to Section 8.9.2 for details of the reporting system.

Outcome of any non-serious AE occurring within 30 days post-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).

8.9 Prompt Reporting of Serious Adverse Events to the Sponsor

The paper SAE Report Forms and the facsimile (fax)/e-mail system will be the primary modes for reporting SAEs to the Sponsor during the study period. SAEs should also be reported on the SAE screens in the electronic system.

8.9.1 Time frames for submitting serious adverse event reports to the Sponsor

SAEs will be reported promptly to the Sponsor once the investigator determines that the event meets the protocol definition of an SAE. The investigator or designate will complete and submit relevant information on the paper SAE Report Forms and fax or e-mail the (scanned) SAE reports to the Sponsor **WITHIN 24 HOURS OF HIS/HER BECOMING AWARE OF THESE EVENTS**. Additional or follow-up information relating to the initial SAE report is also to be completed and submitted on the paper SAE Report Forms and faxed/e-mailed to the Sponsor within 24 hours of receipt of such information.
During the study, the investigator should also enter and update the SAE information in the SAE screens in eCRF.

8.9.2 Completion and transmission of serious adverse event reports to the Sponsor

Once an investigator becomes aware that a SAE has occurred in a study patient (including a screened patient), the investigator or designate will complete and submit the information on the paper SAE Report Forms and fax/e-mail the SAE reports to the Sponsor within 24 hours as outlined in Section 8.9.1. The paper SAE Report Forms will always be completed as thoroughly as possible with all available details of the event, signed by the investigator or designate and faxed to the Sponsor. If the investigator or designate does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying the Sponsor of the event and faxing the SAE Report Forms. The SAE Report Forms should be updated when additional information is received and faxed/e-mailed to the Sponsor **WITHIN 24 HOURS** as outlined in Section 8.9.1.

The investigator will always provide an assessment of causality at the time of the initial report as described in Section 8.7.2.
NB. An event which is part of the natural course of the disease under study (i.e., disease recurrence) is captured in the study as an efficacy measure; therefore it does not need to be reported as an SAE (see Section 8.4).

In rare circumstances, in the absence of facsimile/e-mail equipment for reporting SAEs ***(including deaths determined by the investigator to be related to treatment administration)***, notification by telephone is acceptable, with a copy of the SAE Report Form sent by mail. Initial notification via the telephone does not replace the need for the investigator or designate to complete and sign the SAE Report Forms and fax/e-mail the SAE form to the Sponsor.

In the event of a death determined by the investigator to be related to study treatment administration, sending of the fax must be accompanied by telephone call to the Study Contact for Reporting SAEs.

Study Contact for Reporting SAEs:

Sponsor: EAU RF: Fax: + 31 26 389 06 79 e-mail address: rfsafety@uroweb.org
Tel: + 31 26 389 06 77

8.10 Regulatory Reporting Requirements for Serious Adverse Events

The investigator will promptly report all SAEs to the Sponsor in accordance with the procedures detailed in Section 8.9. The Sponsor has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority 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.

The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to the IRB/IEC and, if required, to the applicable government authority.

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

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will file it with the Investigator Brochure or other appropriate study documentation and will notify the IRB or IEC, if appropriate according to local requirements.

8.11 Post-study Adverse Events and Serious Adverse Events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE detection period defined in Section 8.6. 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. The investigator or designate should use the paper SAE Report Forms and the facsimile (fax)/e-mail system if SAE follow ups or new SAEs have to be reported.

8.12 Pregnancy

Patients who become pregnant during the study treatment phase must not receive additional doses of study treatment 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 i.e. from signature of first informed consent till the concluding visit , or till recurrence/death if occurring before the the concluding visit . The investigator, or his/her designee, will record pregnancy information on the Pregnancy Report Form and submit it to the Sponsor 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 the Sponsor. Generally, follow-up will be no longer than 6 to 8 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 Section 8.1 and 8.2, and will be followed as described in Section 8.8.

A spontaneous abortion is always considered to be a SAE and will be reported as described in Section 8.8. Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered reasonably related in time to receipt of the investigational product by the investigator, will be reported to the Sponsor as described in Section 8.11. 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 the screening phase/prior to treatment administration does not need to be collected; this information need not be administered in the eCRF.

8.13 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 6.6.

9. PATIENT COMPLETION AND WITHDRAWAL

9.1 Patient Completion

A patient will be considered to have completed the study treatment when he/she has been under treatment till Week 112 (= the end of all the scheduled study visits defined as the concluding visit) following first study treatment administration. The term "study activity" refers to physical visits and does not include follow-up contacts (i.e. phone contacts).
Treatment with investigational treatment (ASCI or placebo) should be conducted and completed according to this protocol unless it has to be ended prematurely because of documented disease recurrence, withdrawal due to an unacceptable toxicity, or withdrawal for other reasons (see Section 9.2).
Once the patient has been withdrawn from investigational treatment or has completed treatment, the reason for cessation of treatment must be documented in the patient's medical records and eCRF.
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9.2 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 an SAE/AE until resolution of the event (see Section 8.8).
Withdrawals will not be replaced.

9.2.1 Patient withdrawal from study product administration

A 'withdrawal' from the study product(s) 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 study product(s) may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed as planned in this study protocol.
The investigator will document in the eCRF whether the decision to discontinue further treatment was made by the patient or the investigator and which of the following possible reasons was responsible for withdrawal:

- (Serious) adverse event (including intercurrent illness, unacceptable toxicity),
- Disease progression/recurrence,
- Protocol violation (specify),
- Other, eg. the occurrence of a second tumor that needs therapy (specify).

In this case, the concluding visit will be performed as described in Section 7.3.2.

If the patient refuses or cannot undergo these study procedures, he/she will be considered as withdrawn from study (Section 9.2.2).

9.2.2 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 activity 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 and in the best interest of the patient, 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 study treatment) will occur.

Information relative to the withdrawal will be documented on the Study Conclusion page of the eCRF. The investigator will document whether the decision to withdraw from the study was made by the patients or the investigator and which of the following possible reasons was responsible for withdrawal:

- 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,
- Other (specify).

9.3 Screen and Baseline Failures

A patient was considered to be a screen/baseline failure if the patient signs the ICF, but withdrew before receiving study treatment.

All patients having given consent to undergo MAGE-A3 expression screening upon resection of their tumor were referenced into a screening section of the eCRF, including patients with a negative MAGE-A3 expression.

10. STATISTICAL CONSIDERATIONS

10.1 Sample Size Calculation

The assumptions for the sample size calculations were the following.

- Primary endpoint on which sample size is based is disease free survival (see Section 3.3)
- Hazard ratio of expected disease free survival of recMAGE-A3 + AS 15 ASCI treated patients relative to placebo treated patients that will be significant at the end of study is 0,75.
- Median Disease-Free Survival in the placebo groups is 24 months
- Recruitment period is 4-5 years
- All patients will be observed for a maximum of 5 years from their first study administration and with a minimum of 6 months after treatment completion.
- Nulhypothesis H0: "there are no differences in expected disease free survival between the placebo treated patients and recMAGE-A3 + AS 15 ASCI treated patients.
- Alternative hypothesis H1: "the recMAGE-A3 + AS 15 ASCI treated patients have a different disease free survival compared to the placebo treated patients.
- Anticipated drop out percentage is 15 %
- One-sided alpha for final analysis is 0,05
- Power to detect a HR= 0,75 is 50 %. Power to detect a HR = 0,65 is 80 %.
- Randomization: 2 recMAGE-A3 + AS 15 ASCI patients versus 1 Placebo patient.
- Interim analysis planned when 65% of the total number of events have occurred.
- Calculations were done using East 5.3.

Based on these assumptions and in order to reach 155 events* (see section 10.2), we needed a total of 273 patients for randomization. A total of 182 patients were to be randomized for treatment with recMAGE-A3 + AS 15 ASCI and 91 patients were to be randomized for treatment with placebo. Total number of evaluable patients (15 % drop out percentage) would be 231.
The power to detect a HR=0.75 (from 24 to 32 months) was 50%. The study would have 65% power if the true HR=0.7 (from 24 to 34 months) and 80% power if the true HR=0.65 (from 24 to 37 months).
Due to the Protocol Amendment 4.0, not all analyses described in this section will be performed and no interim analysis will be done. Please see Section 2.6.2 for the rationale.

* 147 events without interim analysis

10.2 Study Populations to be evaluated

Total Treated population:
The Total Treated population will include all treated patients, i.e. all patients with at least one documented dose of study treatment (ASCI or placebo).
The total treated population will be used for the primary analysis of efficacy.

According To Protocol (ATP) population for analysis of immunogenicity
The ATP population for analysis of immunogenicity will include all evaluable patients (i.e. those meeting all eligibility criteria, complying with the procedures and intervals defined in the protocol, with no elimination criteria during the study) who have received at least the 4 first study treatment doses and for whom data concerning immunogenicity post-dose 4 are available.

According To Protocol (ATP) population for analysis of efficacy
The ATP population for analysis of efficacy will include all evaluable patients (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) who have received documented administration of at least the 4 first doses of study treatment and for whom data concerning efficacy endpoint measures are available.

Elimination criteria for analysis
As defined in Section 6.6.2, certain medications are prohibited during study. Among these medications, the following will not require withdrawal of the patient from the study but may determine a patient evaluability in the according-to-protocol (ATP) analysis.

- Administration of immunosuppressants or other immune-modifying drugs during the study period. The use of prednisone, or equivalent, < 0,125 mg/kg/day (absolute maximum 10 mg/day), or inhaled corticosteroids or topical steroids is permitted.
- Administration of a vaccine not foreseen by the study protocol during the period within the prohibited time as specified in Section 6.6.3.
- Administration of immunoglobulins and/or any blood products during the study period.
- Occurrence of a major protocol violation as indicated in Section 7.1.

10.3 Statistical Analyses

As of Protocol Amendment 4.0, statistical analyses will not be assessed as planned. All clinical data collected in the study will be analysed descriptively. Please see Section 2.6.2 for the rationale.

Patient characteristics, demographics and baseline measurements will be summarized in order to provide a characterization of the patient population. Descriptive statistics, e.g. mean, standard deviation, median, range, frequency distributions as appropriate will be presented for each randomization group: recMAGE-A3 + AS 15 ASCI versus the Placebo arm.

A yearly safety analysis will be performed until all patients have finished the treatment phase (Visit 14) which is 112 weeks after the last patient was randomized. The incidence and severity of all adverse events will be tabulated per treatment group. Treatment related toxicity > grade 2 will be descriptively described in both arms.
The description of the safety data will be performed, first globally then for each of the study arms. The safety analysis will be performed in all patients who are randomized and who received at least one Study Treatment Administration.

The efficacy analysis will be performed including all patients who are randomized and who received at least one Study Treatment Administration.

Disease free Survival (Section 3.3) will be estimated by means of the Kaplan Meier method and comparison between treatment groups will be done by means of the log-rank test.
A Cox proportional hazards model will be applied to correct for treatment effects and to assess the influence of prognostic and confounding factors such as T category, Neo-adjuvant Chemotherapy vs. Adjuvant Chemotherapy vs. no chemotherapy, N category, gender, centre, multifocality of bladder carcinoma (Y/N), age (continuous), presence of Carcinoma in Situ (CIS) and gene profile population.

The analyses of other outcome measurements will be analyzed in an exploratory way with methods appropriate for the type of the variable. Both the safety data and the efficacy data will be summarized by appropriate descriptive statistics.
For each patient entering, the reason for discontinuation (e.g. patient decision, urologists decision, lack of efficacy, adverse events) should be clarified. The description of the reason for discontinuation will be performed.

All tests of efficacy hypotheses based on between-treatment comparisons will be performed at the 5 % level of significance, and will be two-sided. Statistical tests based on within-treatment comparisons will be performed at the 5% level of significance and will be two-sided. No adjustments will be made to nominal significance levels to account for multiple comparisons made on the same data.

11. ETHICAL CONSIDERATIONS

11.1 Regulation Statement

This study will be conducted according to the principles of the Declaration of Helsinki (see for the most recent version: www.wma.net) and in accordance with the European and local regulations and applicable guidelines.

11.2 Recruitment / Consent and Ethical Review Procedure

Eligible patients will be fully informed by the principal investigator (PI), the local investigator and/or the local research coordinator and his research staff, whatever is applicable, about the study and asked to participate. The patient will receive a patient information sheet and will have ample opportunity to ask any question he/she might have. He/she will have sufficient time to consider the study's implications before deciding to participate in the study. Patient's consent will be noted on an informed consent form (ICF).
If during the study the patient for whatever reason no longer wishes to participate he/she can withdraw his/her consent at any time, without any further consequences regarding their treatment.

Submission of the protocol and any protocol amendments to regulatory agencies will occur in accordance with local regulatory requirements.
Prior to the start of the study, in some countries, the protocol has to be approved by one recognized medical ethics review committee for all participating institutions (the accredited review committee). The EC shall form a conclusion on the scientific and medical-ethical aspects of the protocol. If the EC requires further information to form its decision or believes the protocol needs to be adjusted, it shall inform the PI or the Sponsor immediately. On the basis of the additional information or the adjusted protocol, the EC shall reach its conclusion about whether or not the protocol is acceptable in terms of the research's scientific and medical-ethical aspects. Approval will be indicated in writing with reference to the final protocol number, version and date. The use of medication as described in this protocol must not under any circumstances deviate from the agreed protocol. In exceptional circumstances, for example when the health of the subjects is as risk, the investigator can use his clinical judgement and alterations may be made. The event must then be documented in detail to the Sponsor and, if applicable, the EC and the PI and the investigators should be notified.
If in the opinion of the local investigator or the PI the clinical observations in the study suggest it may be unwise to continue, they will be able to terminate the study locally or entirely, respectively.
If it becomes apparent that patient enrolment is unsatisfactory or the quantity or quality of the data received is inaccurate or incomplete on a chronic basis, the PI has the right to terminate the study

and remove all study equipment from the investigational site. If the study is stopped early the EC should be informed about the reasons.

11.3 Benefit and Risks Assessment

Given the situation of the patient who needs a cystectomy, the extra burden and risks associated with participation in the study is considered minimal and acceptable. During the first 3 months in the study, the number of patient visits is higher. This is due to the need for administration of treatment every 3 weeks. Hereafter, the number of visits and treatments is equal to what patients with these criteria is offered when they are treated in the standard way, which is watchful waiting after cystectomy. Possible extra procedures in this study depend on local routine practices. For example 3-monthly CT scans /MRI's can be extra if 6-monthly imaging procedures are considered standard routine practice (during treatment phase only). Also laboratory assessments and the questionnaire on smoking habits can be considered as extra. The risks related to the expected treatment outcome, quality and quantity of side effects of the study medication can be considered as acceptable (see Section 2.5.3). Benefit for the patient is that the patient is followed according to the latest standards and guidelines of treatment of MIBC and the patient has a chance on early detection of recurrence of disease.

11.4 Compensation for Injury

The Sponsor will arrange a liability insurance which is in accordance with GCP and local legal requirements.

12. ADMINISTRATIVE ASPECTS AND PUBLICATION

12.1 Study Organisation & Management

For this multicentre study, an initiating investigators meeting at a central location will be held to standardize data management procedures and resolve questions regarding protocol conduct. At the initiation meeting, the investigator and eventual other site study personnel will be instructed how to conduct the study- and data management procedures. In addition, the sites will be able to download the required materials via the EAU RF website. EAU RF's representatives will conduct field data review periodically. It is the responsibility of EAU RF's representatives to verify adherence to the protocol and the completeness, accuracy and consistency of the data. The investigator or a qualified employee will enter all relevant data into eCRF's, in accordance with the instructions provided. The investigator may be requested to provide a copy of the applicable pathology or radiology reports. These copies will be used as source verification. An explanation for the omission of any required data should appear on the appropriate e-page. The investigator must sign an agreement thereby stating that she/he takes responsibility for the accuracy of the data in the entire eCRF.
Original subject records (e.g., hospital charts, clinical records, laboratory printouts) should be available at each study site for source document review by EAU RF's representatives. Source document review is the cross checking of information recorded on eCRF's with that recorded in the original subject records. It is not the purpose of source document review to ensure that all information in the eCRF is also recorded elsewhere in the subject's records; the purpose is to help ensure that the eCRF accurately reflects information generated during the study. In this study, source document review of specific types of information will be conducted in all patients enrolled. EAU RF's representatives ensure the privacy of the patient data by only collecting the patient data without the patient details that could identify the individual patient. The investigator should give the monitor access to all relevant patient data.
Queries to be issued to the investigator will consist of questions to clarify for instance missing data, inconsistencies, illegible data, illegal values and items that are not clearly corrected.
When all patient and visit data are received at EAU RF, all data problems have been resolved, all data checks and quality control checks have been performed, the study database is considered to be clean and can be locked. This cleaning and locking process will be performed on a per patient and per visit basis. In addition, the study centre may be audited in depth for study quality assurance by EAU RF representatives, and/or inspected by a national regulatory authority. This audit may include review of all source documents, drug records, original clinic case-notes, some or all of the

facilities used in the trial, etc. Patient confidentiality will be maintained at all times and consent for this will be obtained prior to entry of the patient into the clinical trial.

12.2 Participating Local Investigator

The participating local investigator is authorised to randomize patients in this study as soon as EC and CA approval has been obtained and, depending on local regulations, approval has been obtained from the managing board or the board of directors of the hospital. It is the responsibility of the investigator at the local site to verify adherence to the protocol, the protection of the rights of the patient, the completeness, accuracy and consistency of the data to be entered by eCRF and adherence to local regulations.

12.3 Principal Investigator

The principal investigator (PI), representing the Sponsor EAU RF, is responsible for protocol writing and case report form design, patient registration and assigning patient sequential numbers, handling of serious or unexpected adverse events reports, performing consistency checks of the case report forms, issuing queries in case of inconsistencies, reviewing and confirming all objective tumor responses and the preparation of the manuscript for publication. Some of these responsibilities will be handled by EAU CRO on behalf of the Sponsor/PI.

12.4 EAU CRO

EAU CRO will assist the Sponsor/PI in protocol writing, eCRF design, patient registration and assigning patient sequential numbers, performing consistency checks of the case report forms, issuing queries in case of inconsistencies and the preparation of the manuscript for publication. On behalf of the PI and the Sponsor, EAU CRO will be also be responsible for initiating and monitoring the study, supply of eCRF's, randomization of patients, query management, statistical analyses, the preparation of the study report and overall study-management.

12.5 Amendments

Amendments are changes made to the research protocol after a favourable opinion by the CA/ EC has been given.

A 'substantial amendment' is defined as an amendment to the terms of the EC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:
the safety or physical or mental integrity of the subjects of the trial; the scientific value of the trial; the conduct or management of the trial; or the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the EC that gave a favourable opinion. Written IRB/EC approval of substantial amendments is required prior to implementation.
Non-substantial amendments will not be notified to the EC, but will be recorded and filed by the sponsor. Examples of non-substantial amendments are typing errors and administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted study documentation.
Considering the long duration of this trial it is foreseen that participating sites may discontinue their participation, new sites will be added or that the coordinating investigator of individual sites changes. Any of these changes will not result in a substantial protocol amendment if the reasons for these changes are of a logistic nature only and do not jeopardise the overall conduct of this study.

12.6 Annual Progress Report

The Sponsor/PI will submit a summary of the progress of the trial to the EC once a year.
Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ suspected serious adverse reactions (SUSARs), other problems, and amendments.

12.7 End of Study Report

The Sponsor/PI will notify the EC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
In case the study is ended prematurely, the Sponsor/PI will notify the EC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the Sponsor/PI will submit a final study report with the results of the study, including any publications/abstracts of the study, to the EC.

12.8 Public Disclosure and Publication Policy

Any formal presentation or publication of data collected from this trial will be considered as a joint publication by the investigator(s) on behalf of the sponsor. The PI and the steering committee members will be authors on presentations/publications. Every participating site should designate one member to be author on presentations/publications. Further authorship will be determined according to the number of eligible patients enrolled and the quality of the patients' follow up at each participating site.
For multi-center studies, it is mandatory that the first publication is based on data from all centers, analysed as stipulated in the protocol by epidemiologists/statisticians in conjunction with the PI,

and not by the investigators themselves. Investigators participating in this multi-center study agree not to present data gathered from one center or a small group of centers before the full, initial publication, unless formally agreed to by the Sponsor/PI.

The Sponsor/PI must receive copies of any intended communication in advance of publication (at least 15 working days for an abstract or oral presentation and 45 working days for a journal submission). The Sponsor/PI will review the communications for accuracy, verify that confidential information is not being inadvertently divulged and to provide any relevant supplementary information.

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Appendix 1: Protocol Signature Sheet

Investigator Signature:

I have read and agree to the **'A randomized, double-blind, placebo controlled phase II trial to evaluate the safety and efficacy of recMAGE-A3 + AS 15 ASCI in patients with MAGE-A3 positive muscle invasive bladder cancer after cystectomy. A European Association of Urology Research Foundation Randomized Phase II Clinical Trial, Final version 4.1, October 28th, 2014.**

I am aware of my responsibilities as an Investigator under the guidelines of Good Clinical Practice (GCP), local regulations (as applicable) and the study protocol. I agree to conduct the study according to these guidelines and to appropriately direct and assist the staff under my control, who will be involved in the study.

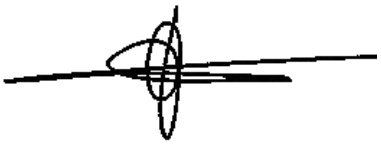
NAME:
TITLE: INVESTIGATOR: SIGNATURE: _____

DATE: _____

PLACE:

Full investigational site contact details, including telephone numbers, will be documented in the Trial Master File.

Principal Investigator:

NAME: Prof. Peter F.A. Mulders SIGNATURE: 

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DATE: October 28th, 2014

PLACE: NIJMEGEN

Appendix 2: WHO Performance Status

WHO Performance Status

The WHO score (published by Oken *et al* in 1982 (Ref. 21), also called the ECOG or Zubrod score (after C. Gordon Zubrod), runs from 0 to 5, with 0 denoting perfect health and 5 death:

0. Asymptomatic
1. Symptomatic but completely ambulatory
2. Symptomatic, <50% in bed during the day
3. Symptomatic, >50% in bed, but not bedbound
4. Bedbound
5. Death

Appendix 3: TNM classification

3.1 Tumour, Nodes, Metastases Classification (TNM)

The 2002 TNM classification approved by the Union International Contre le Cancer (UICC) has been widely accepted (Table 1) (5). It differs from the previous versions in the definition of stage T2 and T3 tumours.

Table 1: 2002 TNM classification of urinary bladder cancer

T - Primary tumour		
TX	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
	Ta	Non-invasive papillary carcinoma
	Tis	Carcinoma in situ: 'flat tumour'
T1	Tumour invades subepithelial connective tissue	
T2	Tumour invades muscle	
	T2a	Tumour invades superficial muscle (inner half)
	T2b	Tumour invades deep muscle (outer half)
T3	Tumour invades perivesical tissue:	
	T3a	Microscopically
	T3b	Macroscopically (extravesical mass)
T4	Tumour invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall	
	T4a	Tumour invades prostate, uterus or vagina
	T4b	Tumour invades pelvic wall or abdominal wall
N - Lymph nodes		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Metastasis in a single lymph node 2 cm or less in greatest dimension	
N2	Metastasis in a single lymph node more than 2 cm but not more than 5 cm in greatest dimension, or multiple lymph nodes, none more than 5 cm in greatest dimension	
N3	Metastasis in a lymph node more than 5 cm in greatest dimension	
M - Distant metastasis		
MX	Distant metastasis cannot be assessed	
M0	No distant metastasis	
M1	Distant metastasis	

3.2 Histological grading of non-muscle invasive bladder tumours

In 1998, the new classification of non-invasive urothelial tumours was proposed by the World Health Organization (WHO) and the International Society of Urological Pathology (ISUP) (1998 WHO/ISUP classification) and published by the WHO in 2004 (6,7) (Table 2). Its major contribution is a detailed histological description of the various grades, employing specific cytological and architectural criteria. A website (www.pathology.jhu.edu/bladder) illustrating examples of various grades was developed to improve accuracy further in using the system.

Table 2: WHO grading in 1973 and in 2004 (6,7)

1973 WHO grading	
Urothelial papilloma	
Grade 1:	well differentiated
Grade 2:	moderately differentiated
Grade 3:	poorly differentiated
2004 WHO grading	
Urothelial papilloma	
Papillary urothelial neoplasm of low malignant potential (PUNLMP)	
Low-grade papillary urothelial carcinoma	
High-grade papillary urothelial carcinoma	

Appendix 4: Common Terminology Criteria for Adverse Events v4.0 (CTCAE)

The Common Terminology Criteria for Adverse Events can be used via the internet:

http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx **GO TO LINK** CTCAE v4.0

This is a handy search engine in which the adverse event can be searched and graded easily.

On request, EAU CRO will provide the participating sites with the CTCAE 72 pages pdf file or a paper version, if needed.