Final Study Report

Study Title: Phase I trial of stereotactic body radiotherapy with concurrent pembrolizumab in metastatic urothelial cancer.

EudraCT number: 2016-001263-37

Eudamed number: NA

Study protocol code: 2016-001263-37

ClinicalTrial.gov identifier: NCT02826564

Sponsor: UZ Ghent

National Coordinator/ Coordinating Investigator: Prof Dr Piet Ost

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Date of report: 02/01/2020

Name and signature Sponsor: Piet Ost 

Date signature Sponsor: ……30/3/2020……

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1. Introduction

Metastatic urothelial cancer remains a disease that is associated with few therapeutic options, poor prognosis and short-term survival. Approximately 380 000 new cases are diagnosed each year worldwide, resulting in about 150 000 deaths.

Cisplatin-containing combination therapy is standard for metastatic patients, with a median overall survival (OS) of 13 -14 months and long-term disease-free survival (DFS) in around 7% of patients. Unfortunately, about 50% of patient are unfit for cisplatin-containing chemotherapy and may only be palliated with carboplatin-based regimens, without improvement in OS and PFS. In addition, there is currently no standard second-line therapy for patients who progress following platinum-based therapy (ESMO guidelines 2014) (Kim et al Curr Opin Oncol 2015) (Bellmut et al Annals of Oncology 2015).

Pembrolizumab has been shown encouraging responses in this group of patients, with an overall response rate of 27%. Patients responding to pembrolizumab typically have tumors with elevated PD -1 and PD-L1 expression and are infiltrated by CD8+ cytotoxic T cells (Tumeh et al). These carcinomas are referred to as PD-L1+ TIL+ carcinomas.
Unfortunately, there are still a substantial number of patients that do not respond to pembrolizumab treatment, typically patients with low levels of tumor-infiltrating CD8+ T cells and no signs of T cell activation (J Clin Oncol 31. 2013 (suppl; abstr 3001)). These tumors are typed PD-L1– TIL- (Clin Cancer Res. 2013 March 1; 19(5): 1021–1034.). It is hypothesized that in these non-responding patients, the tumor microenvironment might hinder T cell infiltration and induction of local endogenous immune responses.
Radiotherapy might create a more permissive tumor microenvironment, increasing response rates as radiotherapy increases PD-L1 expression on tumor cells (The Journal of Clinical Investigation 2014 December 17; 124) and stimulates the accumulation and activation of CD8+ T cells (Cancer Immunol Immunother. 2014; 63), all markers for response. Preclinical evidence clearly indicates that combining radiotherapy with anti-PD-1 treatment increases the anti-tumoral activity of both treatments (Cancer Immunol Res. 2014 Dec 19; J Clin Invest. 2014 Feb 3;124) and even produces long -term survival (Int J Radiat Oncol Biol Phys. 2013). These positive effects of radiotherapy are most often observed using high-dose per fraction radiotherapy (>5 Gy per fraction), which can be delivered safely in patients using stereotactic body radiotherapy (SBRT).

The timing of SBRT relative to immunotherapy is of the utmost importance. Unfortunately, the effect of timing has not been examined thoroughly (Front Oncol. 2014; 4). It should be considered that SBRT might increase the risk of immune related adverse events. Therefore, a phase I trial, assessing the safety of this novel combination, is essential. In addition, the timing of SBRT might influence the induction of antitumor immunity. Radiotherapy might stimulate the induction of local endogenous immune responses by pembrolizumab (Molecular oncology 2014, Kelderman; Journal of immunology 2012; 189). Conversely, active immune stimulation by pembrolizumab within the tumor microenvironment might maximize radiation-induced antitumor immunity (J Clin Invest. Jul 1, 2013; 123).

The goal of the proposed research project is to assess the safety (dose limiting toxicity) of the combination of pembrolizumab and high-dose SBRT in patients with metastatic urothelial cancer in a phase I trial. Both the SBRT dose and pembrolizumab will be fixed, but the timing of the combination will be varied. Secondary objectives include response rates and immunologic responses next to local control and PFS. The combination sequence with the most promising response rates and the best safety profile will be selected to continue in Phase II.

2. Objectives of the study

2.1 Primary objectives

Objective: To determine the SBRT-schedule associated with DLT in 20% of patients. Toxicities will be considered as DLT if occurring between the start of SBRT and 12 weeks after completion of SBRT.

Hypothesis: Pembrolizumab can be safely combined with SBRT. Given the complex balance between proliferating tumor cells and diverse immune cells, we hypothesize that different timing of SBRT might have a different risk of immune related adverse reactions. However, there are no data available yet on the safety of the proposed combination.

2.2 Secondary objectives

(1) Objective: To assess response of the combination treatment in non-irradiated metastases.

Hypothesis: Based on our knowledge on the role of the radiotherapy in anti-tumor immunity (De Wolf et al. OncoImmunology 2015), and based on the importance of specific immune related biomarkers for response to PD-1/PD-L1 targeting agents, we hypothesize that combining pembrolizumab with SBRT (Cancer Immunol Res. 2014 Dec 19; J Clin Invest. 2014 Feb 3;124), will increase response rates compared to either treatment alone. Response will be evaluated at day 84 (+ /- 7 days) of the study.

(2) Objective: To determine local control of the irradiated metastases.

Hypothesis: Preclinical evidence clearly indicates that combining radiotherapy with anti-PD-1 treatment increases the anti-tumoral activity of both treatments (Cancer Immunol Res. 2014 Dec 19; J Clin Invest. 2014 Feb 3;124). We hypothesize that the combination treatment will increase local control of the irradiated lesions and that pembrolizumab might act as a radiosensitiser.

(3) Objective: To assess progression-free survival and overall survival.

Hypothesis: Preclinical evidence demonstrates that combining radiotherapy with anti-PD-1 treatment not only increases the anti-tumoral activity of both treatments (Cancer Immunol Res. 2014 Dec 19; J Clin Invest. 2014 Feb 3;124) but also produces long-term survival (Int J Radiat Oncol Biol Phys. 2013). We hypothesize that the combination treatment will result in increased progression free survival and overall survival compared to pembrolizumab in monotherapy.

2.3 Exploratory Objective

Objective: To evaluate response rates in PD-L1 TIL- tumors.

Hypothesis: Since radiotherapy has been observed to increase PD-L1 expression and stimulate CD8+ T cells and Th1 cells, all markers for response to pembrolizumab treatment, we hypothesize that combining pembrolizumab with SBRT might result in increased response rates of PD-L1- TIL- tumors. The expression of PD-L1 on tumor tissue, from an archival primary tissue sample or a newly obtained biopsy of a metastatic lesion, will be determined at the moment of inclusion, using immunohistochemistry (IHC).

Objective: To evaluate immunologic responses in peripheral blood samples.

Hypothesis: We hypothesize that an immunological response as measured in the peripheral blood might predict the response the response rate and improve patient selection for future clinical applications.

3. Investigational Medicinal Product

Background

For detailed background information on pembrolizumab (MK-3475) we refer to the Summery of Product Characteristics (SmPC))/.

Pharmaceutical and Therapeutic Background of KEYTRUDA® (pembrolizumab)

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (

PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors . Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. KeytrudaTM (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

Rationale for Dose Selection/Regimen/Modification of MK-3475

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels , 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The

differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i. e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

Rationale for Dose Selection/Regimen/Modification of Radiotherapy

The positive effects of radiotherapy on antitumor immunity are most often observed using high-dose per fraction radiotherapy (>5 Gy per fraction) (Deng et al The journal of Clinical Investigation 2014; Gupta et al. Journal of immunology 2012), which can be safely delivered in patients using SBRT (Tree et al. Lancet Oncology 2013). SBRT is an advanced radiotherapy delivery technique that allows for the safe delivery of high-dose per fraction radiation.

Preclinical data suggests that a multifraction approach is more beneficial compared to single fraction SBRT, both in terms of local and distant control. Three fractions of 8 Gy were observed to be more effective than 5 fractions of 6 Gy in inducing anti-tumor immunity, since they cause a significant increase in CD4+ and CD8+ TILs and elevated tumor-specific production of IFN-γ ADDIN EN.CITE (Dewan et al.; Schaue et al). We therefore have opted for the 3x8 Gy schedule delivering a total dose of 24 Gy. Clinical SBRT trials using this dose have been shown to be extremely safe without any grade 3 toxicity ADDIN EN.CITE (Salama et al.).

Simulation:

All patients will receive a CT in supine position with 3 mm CT slice thickness through the tumor site. The planning simulation should cover the target and all organs at risk. A typical scan length should extend at least 10 cm superior and inferior beyond the treatment field borders. Support devices to increase patient comfort will be chosen depending on the tumor localization. Lung and liver tumor sites will be simulated with 4D-CT, taking into account breathing. The isocenter will be determined on the CT-simulator with marking of laser lines on the patient. Imaging data will be transferred to the treatment planning system. The type of organ at risk delineated depends on the localization of the metastasis.

OAR definition and Target definition:

For spinal lesions, a pre-treatment axial MRI is required to assess the extent of disease and position of the cord. This must be fused with the planning CT scan. The spinal cord will be delineated according to the RTOG 0633 protocol with 2 different contour sets and specific constraints: conventional spinal cord and partial spinal cord.

a. Conventional: The conventional spinal cord volume is contoured on the simulation CT based on the image fusion with T2-weighted and T1-weighted MRI with contrast. It is recommended that a simulation CT be done with contrast, but this is not required. The conventional spinal cord should be contoured starting at least 10 cm above the superior extent of the target volume and continuing on every CT slice to at least 10 cm below the inferior extent of the target volume. This spinal cord volume is required to be consistent with image-guided radiotherapy volume definition of RTOG protocols.

b. Partial: The spinal cord is contoured based on the image fusion with T2-weighted and T1-weighted MRI with contrast. It is recommended that a simulation CT image be done with contrast, but this is not required. The partial spinal cord should be contoured starting from 5-6 mm above the superior extent of the target volume to 5-6 mm below the inferior extent of the target volume. The spinal cord should be drawn on every slice of simulation CT. The variation of 5-6 mm is due to the pre-determined slice thicknesses of 2.5-3 mm by different CT manufacturers.

- Gross Target Volume (GTV): all visible tumor by combining iconographic and metabolic information. No clinical target volume will be delineated.

- Planning Target Volume (PTV): expansion from GTV to account for organ motion and setup error. Margins depend on the site irradiated with 2 mm margins for bony lesions and 5 mm for other sites. In case of overlap between OAR and PTV exists, an PTV_optim is created by subtracting the OAR from the PTV volume. This PTV_optim will be used to prescribe the dose instead of the PTV.

- A Planning Organ at Risk Volume (PRV) expansion of 2 mm will be added to the spinal cord, oesophagus, intestine,… (if applicable), and dose constraints apply to this PRV. It is strongly recommended that dose constraints not be exceeded. If a dose constraint cannot be achieved due to overlap of the target with an organ at risk, the fractionation can be increased or the target coverage compromised in order to meet the constraint.

Radiotherapy treatment planning and dose prescription:

IMRT (static or rotational) treatment planning will be dependent the localization of the metastasis. Dose constraints organ at risks will be in accordance with the recommendations from the report of

the AAPM task group 101 (32). The total dose (80% of the maximal dose) will be delivered in 3 fractions and fractions will be separated >48h and <96h (33, 34). Treatment will be prescribed to the periphery of the target (80% of the dose (=30Gy), should cover 90% of the PTV) covering the PTV. In case of violation of dose constraints to the surrounding organs at risk, the prescription will be adapted accordingly.

Radiotherapy delivery and verification:

Treatment will be delivered with static or rotational IMRT with 6-18 MV photons of a linear accelerator using cone-beam CT set-up and on-line correction of patient's position. If multiple targets will be irradiated and the targets are more than 10 cm apart in the cranio-caudal direction, multiple isocenters are needed with a CBCT prior to every treatment for every isocenter. Patient immobilization devices can be used according to the institutional policy.

4. Investigational Medical Device

NA

5. Study Protocol Summary

Diagnosis/Condition for Entry into the Trial

Patients with the diagnosis of metastatic urothelial carcinoma are allowed to enter into the trial.

Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.
2. Be ? 18 years of age on day of signing informed consent.
3. Have measurable disease based on RECIST 1.1.
4. Have had any prior treatment more than 2 weeks prior to study day 1, treatment naïve patients are allowed
5. Histologically confirmed diagnosis of urothelial carcinoma
6. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly-obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Sponsor.
7. Have a performance status of 0 or 1 on the ECOG Performance Scale.
8. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 10 days before treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	

	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine OR Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) OR ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN OR
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN OR ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

9. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
10. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

11. Male subjects of childbearing potential (Section 5.7.1) must agree to use an adequate method of contraception as outlined in Section 5.7.1- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
2. Has had radiotherapy interfering with SBRT.

3. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
4. Has a known history of active TB (Bacillus Tuberculosis)

5. Hypersensitivity to pembrolizumab or any of its excipients.
6. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
7. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
9. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
10. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
11. Has known history of, or any evidence of active, non-infectious pneumonitis.
12. Has an active infection requiring systemic therapy.
13. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
14. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
16. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
17. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
18. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
19. Has received a live vaccine within 30 days of planned start of study therapy.

Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines n are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5.1 Primary endpoint

see above

5.2 Secondary endpoints

see above

5.3 Procedures

Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental
Radiotherapy	8 Gy	3 fractions, every other day	External beam radiotherapy	Arm A: day -1, -3, -5 Arm B: day 38, 40, 42	Experimental

Trial treatment should begin on the day of randomization or as close as possible to the date on which treatment is allocated/assigned.

5.4 Randomisation and blinding

Patients will be randomly assigned to a treatment arm. After each cohort of 5 patients, determine the posterior probability of the toxicity rate exceeding 0.2 for each arm (=P(Qi ≥ 0.2|data) with Qi the toxicity rate for arm Ti). If this posterior probability exceeds 0.2, close the treatment arm. See statistical analysis plan for details.

6. Study analysis

6.1 Statistical Analysis Plan Summary

The Department of Radiotherapy at the Ghent University Hospital intends to perform a randomized phase I trial on pembrolizumab combined with radiotherapy in metastatic urothelial cancer. Both therapies have shown to be safe when applied separately (toxicity probabilities are estimated below 10%). The goal is to determine whether the combination of these therapies possibly accumulates severe toxicities and whether the order of the treatments plays a part in this.

Two treatment arms are considered:

- Arm T1: 4 cycles of pembrolizumab with SBRT applied before the first cycle
- Arm T2: 4 cycles of pembrolizumab with SBRT applied before the third cycle

6.2 Statistical Analysis Plan

The proposed designs are based on the following paper and adapted for our purpose:

Huang, X. A Parallel Phase I/II Clinical Trial Design for Combination Therapies. Biometrics 2007; 63: 429-436.

The implementation is performed in the statistical program R (2.15.2). To construct the designs, we assume that both treatment arms are equally toxic and we considered a treatment arm not safe if the toxicity probability exceeds 0.2.

We have considered the following design: Assign patients randomly to a treatment arm. After each cohort of 5 patients, determine the posterior probability of the toxicity rate exceeding 0.2 for each arm (=P(Qi ≥ 0.2|data) with Qi the toxicity rate for arm Ti). If this posterior probability exceeds a certain cutoff, close the treatment arm.

The posterior probability is calculated using a Beta(0.1,0.9) prior and takes into account the uncertainty at the beginning of the trial when the number of patients is small. The cutoff value that is used for the posterior probability is explored in the simulation settings. For each simulation setting, we used 1000 repeats.

We explored the following settings:

- True toxicity probabilities for both arms: - 0.1, 0.15, 0.17, 0.19 ('Type I' settings) - 0.2, 0.25, 0.3, 0.35 ('Power' settings)
- Cutoff values: 0.8, 0.85, 0.9, 0.95
- Sample sizes (both arms together): 20, 30, 60

A sample of 20 patients and a cutoff value of 0.85 seems sufficient to make an accurate decision about the safety of both treatment arms.

6.3 Statistical Analysis Plan Secondary end points

1. Assess response of the combination treatment in non-irradiated metastases.

The secondary end point is to assess the response in non-irradiated metastases in every treatment arm. The null hypothesis that the true response rate is 0.21 (Bellmunt et al. New England Journal of Medicine 2017; Sharma et al. The Lancet Oncology 2016; Sharma et al. The Lancet Oncology 2017) will be tested against a one-sided alternative. If there are 2 or fewer responses, the alternative hypothesis will be rejected. Otherwise 13 additional patients will be accrued for a phase II trial. The null hypothesis will be rejected if 7 or more responses are observed in 23 patients. This design yields a type I error rate of 0.15 and power of 0.8 when the true response rate is 0.41.

Simon's 2-stage Optimum design will be used.

Alpha	0.15
Power	0.8
Response Probability of pembrolizumab only (P0)	0.21
Response Probability of pembrolizumab in combination with SBRT (P1)	0.41

Optimal Two Stage Design	Optimum Design
First Stage Sample Size (n1)	10
r1	2

Maximum Sample Size (n)	23
r2	6

2. Determine local control of the irradiated metastases.

Descriptive statistics will be provided.

3. Assess progression-free survival.

PFS will be defined as the time from inclusion to documented disease progression according to irRC or death from any cause. Descriptive statistics based on the Kaplan-Meier will be provided.

7. Independent Ethics Committee and Competent Authority

The study protocol was approved by the Ethics Committee of Ghent University Hospital (EC2016/0661).

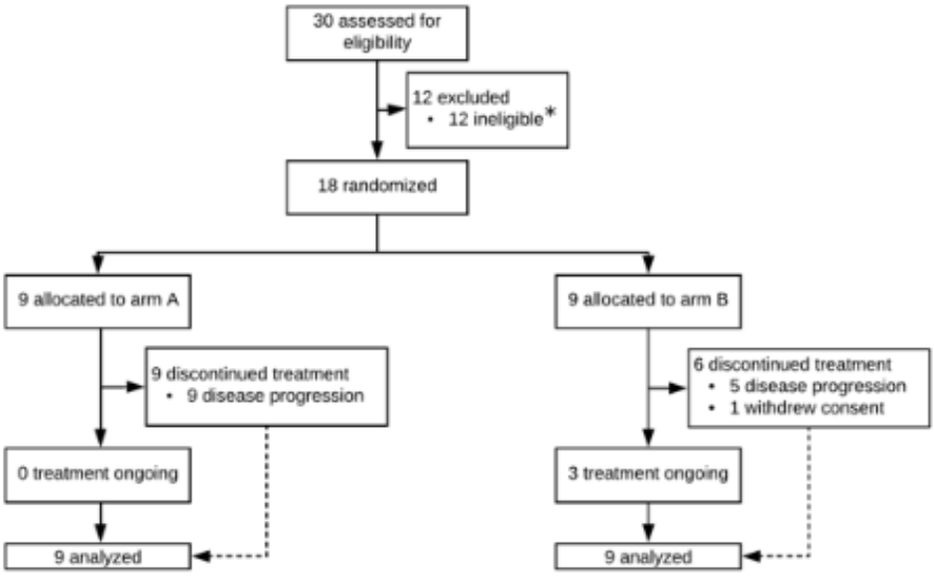
OVERVIEW APPROVED DOCUMENTS		
Initial submission: - Protocol version 1.0, dd. 2/05/16 - ICF v1.0	Approval date Central EC: 7/6/16	Approval date FAGG: 11/07/16 FAMHP: 18/7/16
Amendment 1: - see letter	Approval date Central EC: 09/08/16	Approval date FAMPH: 10/8/16
Amendment 2: - see letter - Protocol dd 07/12/2016	Approval date Central EC:31/1/17	
Amendment 3: - see letter - Protocol v2 dd12/4/2017 - ICF v2 dd 12/4/17	Approval date Central EC:26/4/17	
Amendment 4: - see letter	Approval date Central EC:20/6/17	
Amendment 5: - see letter	Approval date Central EC:23/10/17	Approval date FAMPH: 9/11/17

Amendment 6: - see letter	Approval date Central EC:	

8. Results

8.1 Subject enrollment and demographics

See figure and table



Baseline patient characteristics

	Arm A (N=9)	Arm B (N=9)
Age – yr		
Median	58	71
Range	54-75	50-84
Male sex – no (%)	8 (89%)	8 (89%)
ECOG performance-status score – no. (%)		
0	4 (44%)	6 (67%)
1	5 (56%)	3 (33%)
Previous systemic treatments		
0	2 (22%)	3 (33%)
≥1	7 (78%)	6 (67%)
≥2	3 (33%)	1 (11%)
3	2 (22%)	0
Current or former smoker – no. (%)	1 (11%)	1 (11%)
Modified proportion score of PD-L1 ≥1% – no. (%)	3 (33%)	6 (67%)
Modified proportion score of PD-L1 ≥10% – no. (%)	2 (22%)	5 (56%)
Modified proportion score of PD-L1 ≥95% – no. (%)	1 (11%)	2 (22%)
Visceral disease – no. (%)	5 (56%)	6 (67%)
Liver metastases – no. (%)	2 (22%)	1 (11%)
Hemoglobin concentration <10g/dL – no. (%)	0	1 (11%)

Between 14/11/2016 and 2/1/2018, 30 patients with mUC were screened for enrolment. Eighteen patients were enrolled and randomized (nine to each arm) and received at least one dose of pembrolizumab or one fraction of SBRT (Supplementary Fig. 1). Because of slow recruitment and absence of dose-limiting toxicity, an interim analysis was performed on 20/1/2018, after 18 patients had received trial treatment. For the whole cohort, the median follow-up time was 36 weeks (interquartile range [IQR] 16–59) and the median treatment time was 18 weeks (IQR 11-30). No patients were lost to follow-up. All patients received trial-prescribed SBRT at the planned time point.

Last patient last visit: 27/12/18

9. Statistical interim analysis

Intro

This study was designed to include 20 patients on 2 arms to assess toxicity in both arms. A variable length randomized block design is used, where the toxicity is assessed when a newly entered patient is the first of a new block. This assessment is performed on the weighted toxicity-status (weighted by time since treatment vs planned 84 days of follow up), to allow for staggered entry without having to wait for a complete block of patients having run through the complete 84 days of follow up.

The number of observed toxicities and the weighted number of patients without observed toxicities is combined with a beta-prior (). This prior reflects the prior estimate for the risk of toxicity (0.10) and has the weight of one observation: each new observation has the same weight as the beta-prior. An arm is closed if the posterior probability of the 'risk of toxicity being larger than 0.20', is more than 0. 70 when a new block is started or at the end of the study.

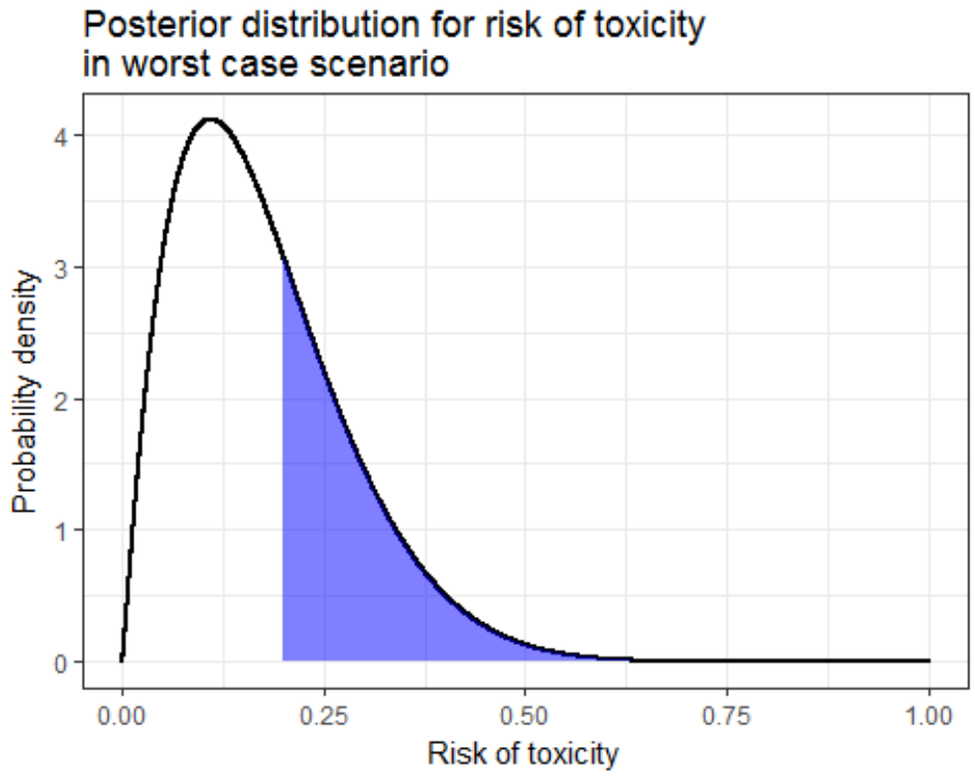
By November 22 2017, 18 patients were accrued. No dose-limiting toxicities were observed in these patients. In each arm, 9 patients were included for analysis. No new patients had been recruited since July 2017, which was probably due to the decision of the national health system (RIZIV/INAMI) to reimburse immunotherapy for these patients. Therefore, a (non-pre-specified) interim analysis was performed on this time point.

This report evaluates the impact that stopping the study early might have on our intended conclusion on safety. To this end a sensitivity analysis is conducted where the worst case scenario poses the question: what would be the outcome if the final two patients would be assigned to one arm and would both experience dose-limiting toxicity?

Arm A

To evaluate this scenario, we combine 11 observations with 2 toxicities, with the beta-prior .

This results in a posterior probability of 0.35 for the risk of toxicity to be larger than 0.2. This is lower than 0.70, so arm 1 would be considered safe. The colored area in the graph below, represents this posterior probability.



Arm 2

As the situation is symmetrical in arm 1 and 2, arm 2 would also be considered safe in the previously specified scenario.

Conclusion

By closing the study early, we do not risk to alter (through the missing observations) a conclusion that would have led to closing one of the treatment arms. Whether or not we reach the full data set, conditional on the current data, we will reach the same conclusion under each possible data augmentation: that there is no evidence of unacceptable toxicity at this stage. Thus, in the given situation of halted accrual, it is acceptable to end the study and present the conclusions based on the available data. Due to the early termination of this first phase, we will not continue to the second phase of this trial.

9.1 Study specific results

See publication and supplementary file

10. Safety

SAE Overview				
Subject ID	Study Arm (if applicable)	SUSAR (Y/N)	SAE Description	Outcome (ongoing, resolved, death, …)
ID12	B	N	auto-immune adrenalitis	resolved
ID2	A	N	Haematuria	resolved
ID8	A	N	lower back pain	resolved
ID8	A	N	Urosepsis	resolved
ID8	A	N	Fever	resolved
ID8	A	N	pulmonary embolism (afterwards noted: due to progressive disease)	death (due to progressive disease)
ID10	B	N	acute kidney injury	resolved
ID13	A	N	hypercalcemia	resolved
ID13	A	N	dyspnea (afterwards noted: due to progressive disease)	death (due to progressive disease)
ID16	A	N	Urinary infection	resolved

11. Device deficiencies

NA

12. Protocol deviations

Subject ID: 17, Date of deviation: 13/4/17, date identified: 6/2/20, type: minor E, classification: minor, description: full physical examination and ECOG not documented where needed, action taken: no action taken.

Subject ID: 17, Date of deviation: 4/5/17, date identified: 6/2/20, type: minor D, classification: minor, description: INR, aPTT and T3 not determined prior to cycle 2, action taken: no action taken.

Subject ID: 17, Date of deviation: 15/6/17, date identified: 6/2/20, type: minor D, classification: minor, description: urine analysis not done at safety evaluation, action taken: no action taken.

13. Discussion and overall conclusions

See publication and supplementary file.

14. References

See publication and supplementary file.