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1.0 TRIAL SUMMARY

Rationale: Despite the early stage of disease, a substantial number of patients with early stage non-small-cell lung cancer (NSCLC) relapse within five years of treatment. A pooled analysis of the ROSEL and STARS trials showed that stereotactic body radiotherapy (SBRT) may provide an alternative to surgical treatment for patients with early stage disease. One of the reasons for the favorable effects of SBRT may be due to an immunological response against tumor cells, elicited by radiotherapy. In this protocol we will explore the addition of immunotherapy to SBRT to augment the antitumor T cell response, associated with radiotherapy. Because surgical resection is still the current standard of care for patients who are fit to undergo surgery, the subjects will undergo a lobectomy with hilar and mediastinal lymph node dissection after SBRT +/- pembrolizumab treatment. This is a key feature of this study and an unique opportunity to perform histological examination of the entire lung tumor, its associated lymph nodes and normal lung tissue after immunotherapy, allowing the study of immune response heterogeneity and correlating this with non-invasive imaging and blood results. In the surrounding normal lung tissue, radiation and immunotherapy induced toxicity can be evaluated.

Objectives: To evaluate the safety and mechanisms of action of the trimodality treatment approach.

Study design: An open label randomized exploratory study of the safety and mechanisms of action of combined treatment with SBRT and immunotherapy (pembrolizumab, anti-PD1) for early stage NSCLC.

Estimated study duration: We expect to enroll one patient per month. Therefore, the study can be finished within 2.5 years from the time the first subject signs the Informed Consent Form (ICF) through the final contact.

Study population: Patients with histologically or cytologically confirmed diagnosis of early stage (T1N0 and T2aN0) peripherally located NSCLC, eligible for surgical resection.

Intervention: Patients will be randomized between SBRT with or without 2 cycles of pembrolizumab treatment (starting on the first day of radiotherapy). The patients will undergo a lobectomy with hilar and mediastinal lymph node dissection after SBRT +/- pembrolizumab treatment. Translational research to explore the immune mechanism of action will include biological imaging with immuno-PET. Expression rates and activation states of immune effector subsets will be assessed in tumor core biopsy specimens, peripheral blood and tumor draining lymph nodes (TDLNs) by means of fine needle aspirates of TDLNs. Samples will be taken before and after SBRT +/- pembrolizumab treatment and at surgery.

Main study parameters/endpoints: To assess the safety of combined SBRT and pembrolizumab treatment in early stage NSCLC and to identify the immunological mechanism of action.

2.0 TRIAL DESIGN

2.1 Trial Design

This is an open label randomized exploratory study of the safety and mechanisms of action of combined treatment with stereotactic body radiotherapy (SBRT) and immunotherapy (pembrolizumab, anti-PD1) for early stage NSCLC. Because the mode of action of combined

SBRT and anti-PD-1 therapy is unknown, it is necessary to perform translational research to identify the mechanisms of action and toxicity before large, more expensive and time consuming effect size studies can be initiated with recurrence free and overall survival as outcome parameters.

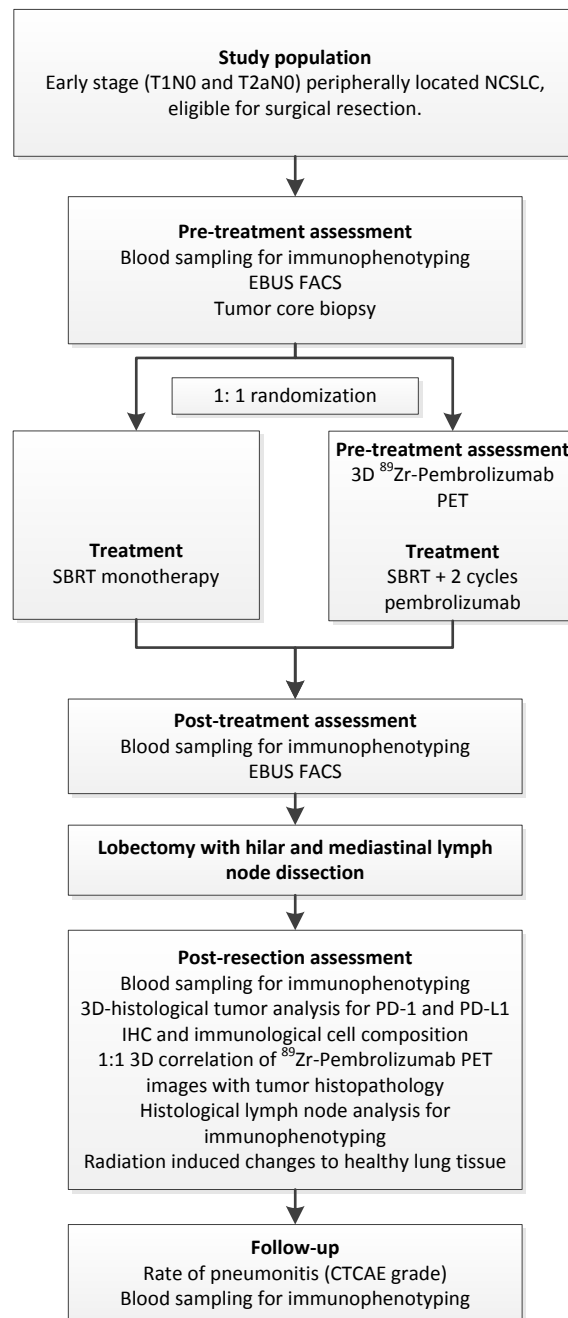
Patients with early stage (T1bN0 and T2aN0) peripherally located and histologically confirmed NSCLC, eligible for surgical resection, will be enrolled in this study. Patients will be randomized between SBRT, with or without 2 cycles of pembrolizumab treatment (starting on the first day of radiotherapy). Because surgical resection is the current standard of care for these patients, the subjects will undergo a lobectomy with hilar and mediastinal lymph node dissection after SBRT +/- pembrolizumab treatment.

Surgical resection after the induction treatment is a unique opportunity to perform histological examination of the entire lung tumor and lymph nodes after immunotherapy, allowing to examine immune response heterogeneity and to correlate this with the non-invasive imaging and blood results. In the surrounding normal lung tissue, radiation and immunotherapy induced toxicity can be evaluated.

In week 8-10 after SBRT +/- pembrolizumab treatment, patients will undergo a lobectomy with hilar and mediastinal lymph node dissection. Patients randomized to 'no pembrolizumab treatment' will also undergo a resection 8-10 weeks after SBRT.

The primary endpoints of this study are safety of the trimodality approach and unraveling the immune mechanism of action. Serial invasive and noninvasive measurements of immunological biomarkers will be performed for the primary tumor, lymph nodes and peripheral blood. A study scheme is provided on page 11 of this protocol. The translational research will include biological imaging, immune effector subset examination and examination of healthy tissue for radiation induced changes.

2.2 Trial Diagram



3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objectives & Hypotheses

Objective 1:

To assess the safety of combined SBRT and pembrolizumab treatment.

Objective 2:

To identify the immunological response to combined SBRT and pembrolizumab treatment in early stage NSCLC. Expression rates and activation states of immune effector subsets will be assessed in tumor core biopsy specimens, peripheral blood and tumor draining lymph nodes (TDLNs) by means of EBUS derived fine needle aspirates. Samples will be taken before and after SBRT +/- pembrolizumab treatment and at surgery.

Hypothesis 2:

We anticipate that SBRT and pembrolizumab treatment will alter the immune activation status as well as the cellular composition in TDLNs, tumor and blood reflected by local and/or systemic switches from a suppressive immune content to a more activated immune content. The magnitude of this response is higher in the combination SBRT and pembrolizumab group than in the SBRT monotherapy group.

3.2 Secondary Objectives & Hypotheses

Objective 1:

To assess uptake (visual and quantitatively, expressed as SUV_{max} , SUV_{mean} and SUV_{peak}) of radiolabeled pembrolizumab (^{89}Zr -pembrolizumab) in the tumor and lymph nodes by use of PET.

Hypothesis 1:

^{89}Zr -pembrolizumab tumor uptake heterogeneity between patients is expected and can be visualized. We expect that the level of ^{89}Zr -Pembrolizumab uptake will be related to the immunohistological results (immune effector subsets with special interest to the expression of PD-1 and PD-L1) of the whole tumor as well as regional tumor areas.

3.3 Explorative Objectives

Objective 1:

To evaluate radiation induced changes to healthy lung tissue after combined SBRT and pembrolizumab treatment in the resected lung lobe.

Objective 2:

To assess radiation induced changes in the tumor cell surface glycoproteins.

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Stereotactic body radiotherapy for the treatment of early stage NSCLC

The current standard treatment for early stage NSCLC is an anatomical surgical resection, with SBRT being reserved for patients not fit to undergo resection. Despite the early stage of disease, 5-year overall survival after surgical resection is only 45-50% due to locoregional as well as distant relapse (1). Although none of the planned randomized studies of surgery versus SBRT have completed accrual, a pooled analysis of our ROSEL study together with the STARS trial from MD Anderson has been published (2). Estimated overall survival at 3 years was 95% (95% CI 85-100) in the SBRT group compared with 79% (64-97) in the surgery group (hazard ratio [HR] 0.14 [95% CI 0.017-1.190], log-rank p=0.037). Recurrence-free survival at 3 years was 86% (95% CI 74-100) in the SBRT group and 80% (95% CI 65-97) in the surgery group (HR 0.69 [95% CI 0.21-2.29], log-rank p=0.54).

This was a very surprising finding because the main presumed advantage of surgery over SBRT is the ipsilateral hilar and mediastinal lymph node dissection with unexpected lymph node (micro)metastases found in 15% of patients (3). The unexpectedly good results with SBRT may be caused by an immunological response against tumor cells, triggered by the radiotherapy. High dose radiation has been shown to induce an antitumor immune response mediated by T-cell regulation, both in the primary tumor and the TDLNs (4, 5). However, despite the promising results of SBRT and this exciting biological mechanism, overall survival was not different between the two modalities in this study. In another study, 676 patients underwent SBRT for early stage lung cancer with a 2-year recurrence rate of 22% and a median overall survival of 41 months (6). Furthermore, of the 124 recurrences, 82 (66%) were distant recurrences.

These results clearly reveal that disease recurrence rates still remain substantial with both modalities, despite their curative intent.

4.1.2 Pharmaceutical and therapeutic treatment with pembrolizumab

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades (7). Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies (8-12). 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) (13, 14). The structure of murine PD-1 has been resolved (15). 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 CD3 ζ , PKC θ and ZAP70 which are involved in the CD3 T-cell signaling cascade (13, 16-18). 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 (19, 20). 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 (21, 22). Expression has also been shown during thymic development on CD4-CD8⁻ (double negative) T-cells as well as subsets of macrophages and dendritic cells (23). 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 (19, 24-26). 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 (19). 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) (27). 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 (previously known as SCH 900475 and MK-3475) 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.

4.1.3 Preclinical and Clinical Trial Data of pembrolizumab treatment

Please see the Investigator's Brochure for Preclinical and Clinical data.

On October 2, 2015, the U.S. Food and Drug Administration granted accelerated approval for pembrolizumab to treat patients with advanced (metastatic) NSCLC whose disease has progressed after other treatments and with tumors that express $\geq 1\%$ PD-L1, as assessed by the companion diagnostic, the PD-L1 IHC 22C3 pharmDx test.

4.1.4 Combination treatment with radiotherapy and immunotherapy

In this protocol we will explore the addition of immunotherapy to SBRT to augment the antitumor T cell response, already associated with radiotherapy.

In different preclinical studies the expression of tumor antigens has been shown to be up-regulated directly after radiotherapy and might therefore lead to changes in the immune response of the body. It is conceivable that radiotherapy treatment acts as an "in situ vaccine" to prime the immune response. Nascent preclinical and early clinical findings have supported this possibility, suggesting that radiation, through its immune-stimulating properties, may be used as a systemic therapy in addition to a means of local tumor control (28-31).

Although radiotherapy results in T cell stimulation and upregulation of tumor antigens, T cells might be still depressed and tumor tolerable due to upregulation of inhibitory co-signals like CTLA-4 and PD-(L)1.

For this reason, anti-PD-L1 treatment can add to ionizing radiation that increases the production and presentation of tumor antigens (32, 33). Other studies showed that the combination of radiation and PD-L1 checkpoint blockade synergistically reduce immunosuppressive MDSCs (34). Systemic responses have been observed in patients with melanoma treated with the combined regimen of anti-CTLA-4 mAb ipilimumab and radiation, suggesting that coupling radiotherapy with immunotherapy may hold promise for inducing powerful effects in human patients (31, 35).

Preclinical studies in murine models have demonstrated efficacy of combination therapy with immunomodulators and radiotherapy (36). Results of a preclinical study of murine intracranial glioma treated with anti-PD-1 mAb plus radiotherapy showed not only long-term survival of the treated mice, but also robust systemic immunologic memory in the surviving mice, as they were able to reject a secondary challenge of glioma cells injected in the flank (37). The combination of radiation plus anti-PD-1 therapy resulted in a median survival to 53 days ($P < 0.05$ by log-rank Mantel-Cox test), and 15% to 40% of mice survived more than 180 days after treatment (37). The combination therapy increased tumor infiltration by CD8+ CTLs and decreased the number of CD4+ Tregs.

On the contrary, median survival periods were similar for control mice (25 days) and mice given only anti-PD-1 mAbs (27 days) or radiation (28 days). In the test of immunologic memory, naïve and long-term surviving mice were injected in the flanks with GL261-luc cells and all 8 naïve mice died from the growth of the challenged glioma cells, whereas mice that received prior treatment with the combined regimen rejected the glioma challenge (37).

In a preclinical study of triple-negative breast cancer, neither anti-PD-1 mAb nor radiation when given alone were effective in a murine model of triple-negative breast cancer. However, the addition of anti-PD-1 mAbs enhanced the curative capacity of radiotherapy and a-CD137 (an agonist antibody for costimulatory molecule 4-1BB) against both established tumors and secondary tumor challenge, indicating that the combined regimen conferred antitumor immune responses and memory (38). Indeed, the combination of a-CD137, anti-PD-1, and radiation showed greater efficacy (40% rejection) than anti-PD-1 or radiation, given alone or in combination (38).

On the basis of the results of these preclinical studies, several clinical trials have been initiated to assess the efficacy of combining anti-PD-1 immunotherapy with radiotherapy.

4.1.5 Time window for the combination of radiotherapy and immunotherapy

In recent preclinical studies, immunotherapy has been administered either a few days prior to, concomitant with, or post radiotherapy. In mouse colon and breast carcinoma models, delaying administration of anti-CTLA-4 mAbs at 2 days after radiotherapy completion can reduce the therapeutic efficacy when compared with 2 days before or on the day of radiotherapy completion, implying that immunotherapy should not be administered too late after RT (39). Dovedi et al. have evaluated three distinct combinatorial schedules where mice bearing colon tumors received a conventional fractionated radiotherapy of 2 Gy \times 5 with administration of anti-PD-L1 mAbs on day 1 of the radiotherapy cycle (schedule A), day 5 of the cycle (schedule B), or 7 days after the completion of radiotherapy (schedule C),

respectively. No significant difference in overall survival is found between schedules A and B. However, sequential treatment with radiotherapy followed by anti-PD-L1 mAbs 7 days later is obviously ineffective for improving overall survival when compared with radiotherapy alone.

4.1.6 Adjuvant Surgical resection

Because surgical resection is the standard of care for operable patients, the subjects will undergo a lobectomy with hilar and mediastinal lymph node dissection after SBRT +/- pembrolizumab treatment. The use of SBRT as neoadjuvant therapy prior to surgery may provide a novel therapeutic opportunity. In oncology, the use of neoadjuvant radiotherapy or chemoradiotherapy prior to surgery has become widespread for several types of cancer, and in many instances improves local control and/or survival compared to surgery alone (40). Neoadjuvant radiotherapy provides several theoretical advantages, including potentially decreasing the rate of positive margins, decreasing the size of the required resection, or by sterilizing the tumor to avoid seeding of circulating tumor cells during surgery (40).

4.1.7 Safety of the trimodality approach

To our knowledge, no study has employed the combination of SBRT, immunotherapy and resection with the goal of maximizing local and distant tumor control.

In modern radiotherapy, image-derived SBRT delivery has improved treatment precision and the radiation-induced toxicity to normal tissues. The toxicity of radiotherapy is generally limited to the irradiated target organ. For example, esophagitis is a complication seen in radiation of locally advanced central lung tumors and mediastinal lymph nodes. Pneumonitis depends on the target volume and is rarely seen with treatment of early stage lung cancer. In current clinical trials, immune-checkpoint blockade therapy presents acceptable toxicity. Even occasional severe toxicity can be managed through treatment interruption or involvement of other immunosuppressors. Compared with CTLA-4, the blockade of PD-1/L1 has less severe ir-AEs. In the ongoing phase I trial with pembrolizumab in NSCLC, 495 patients were treated. Drug-related grades 3–4 adverse events were found in 9.5% of patients with 1.8% developing pneumonitis (41). Considering the acceptable adverse events, the combination of radiotherapy and immune checkpoint inhibitors appears to be feasible in patients.

Combining SBRT with planned surgery appears safe: at least 4 small studies have reported on patients who have undergone surgery for salvage in patients who have recurred after SBRT (42-45). Such surgery is generally well tolerated with a favorable toxicity profile, with only one patient sustaining a major toxicity (fistula requiring further surgery for correction) (44). An ongoing Phase II Canadian trial is accruing patients with early stage NSCLC for treatment with SBRT plus adjuvant surgical resection with a treatment interval of 10 weeks (NCT02136355). Currently there are five patients who underwent a resection, all without complications. In two patients, viable tumor was found in the resection specimen (personal communication, unpublished data).

As only patients with peripheral tumors are eligible for this trial and patients receive a lobectomy according to national guidelines on the treatment for NSCLC, the resection margins (i.e. central bronchial and vascular structures) will not be involved in the radiation target volume. Therefore we do not foresee problems with healing of these structures and do not

anticipate increased risks for a fistula or other surgical complications, beyond those seen with a primary surgical resection. Furthermore, because the irradiated tumor and surrounding lung tissue are resected with the lobectomy, we do not foresee increased toxicity from combined SBRT and pembrolizumab treatment.

4.1.8 Predictive biomarkers of response to radiotherapy +/- pembrolizumab treatment

Because benefit of the addition of pembrolizumab to radiotherapy is expected to occur in a subset of patients, it is of great importance to identify biomarkers that correlate with clinical activity and that can be used to select patients that will benefit from the addition of pembrolizumab treatment.

Selecting those patients that benefit the most is challenging. Until now companion biomarker development has focused on the level of PD-L1 expression by immunohistochemistry. Among 204 patients with NSCLC treated on an expansion cohort phase I trial, tumor PD-L1 expression (defined in IHC as $\geq 1\%$ tumor membrane PD-L1 expression) was present in 86% of the tested patients. For patients with a proportion score (PS, percentage of cells with membranous PD-L1 staining of any intensity) $\geq 50\%$, the objective response rate (ORR) was 45%, while the ORR was 17% and 11% for patients with PS of 1-49% and $< 1\%$, respectively (41). In the phase III KEYNOTE-010 trial evaluating pembrolizumab versus docetaxel for previously treated PD-L1 ($\geq 1\%$) positive advanced NSCLC patients, ORR was 18%, while this was 30% in patients with a proportion score of $\geq 50\%$ (46).

On October 2, 2015, the U.S. Food and Drug Administration granted accelerated approval for pembrolizumab to treat patients with advanced (metastatic) NSCLC whose disease has progressed after other treatments and with tumors that express $\geq 1\%$ PD-L1, as assessed by the companion diagnostic, the PD-L1 IHC 22C3 pharmDx test.

In conclusion, tumor PD-L1 expression seems to be related to response but the signal is not straightforward since responses are seen across the level of PD-L1 expression and even in patients that are PD-L1 negative. Adding to the complexity, the level of tumor PD-L1 expression is variable over time and influenced by several host and environmental factors such as TNM stage, chemotherapy and cytokines like IFN- α (47, 48). PD-L1- tumors can become positive and vice versa (49). Temporal and spatial variation of tumor PD-L1 expression (within and between tumor lesions) might be responsible for its suboptimal predictive value for treatment benefit. Currently, some study protocols require positive tumor PD-L1 staining for inclusion. Looking at the above there seems to be an urgent need to further validate tumor PD-L1 IHC as predictive biomarker, as well as looking at alternatives.

4.2 Rationale

4.2.1 Rationale of the trimodality approach

As stated in section 4.1.1, despite the promising results of SBRT, overall survival was not different between the two modalities and the relapse rate is still substantial. In a study by Senthil et al, 676 patients underwent SBRT for early stage lung cancer with a 2-year recurrence rate of 22% (6). Furthermore, of the 124 recurrences, 82 (66%) were distant recurrences. Looking at the preclinical and clinical data on the synergistic systemic antitumor effect of combination radiotherapy and immunotherapy, there is a strong biological rationale

to study these two modalities together as induction therapy for patients with operable early stage NSCLC.

In this protocol we will explore the addition of immunotherapy to SBRT to augment the antitumor T cell response, already associated with radiotherapy. This exploratory study aims primarily to assess the safety of the trimodality treatment strategy and to identify and characterize the immunological mechanisms of action of combined SBRT and anti-PD-1 treatment. Because the SBRT technique allows to irradiate small tumor volumes with maximal sparing of healthy lung tissue, this is the ideal and safest setting to introduce the combination of pulmonary radiotherapy and immunotherapy in lung cancer. The results of this study can serve as a proof of principle for the immunological effect of thoracic radiotherapy in general and might serve as a vehicle for further development of the combination of radiotherapy and immunotherapy in early stage lung cancer and stage III disease, a setting where radiation pneumonitis is a well-known and feared complication.

Because surgical resection is still the current standard of care for these patients, the subjects will undergo a lobectomy with hilar and mediastinal lymph node dissection after SBRT and pembrolizumab treatment. This is a key feature of this study and an unique opportunity to perform histological examination of the entire lung tumor and lymph nodes after immunotherapy, allowing to examine immune response heterogeneity and to correlate this with the non-invasive imaging and blood results. In the surrounding normal lung tissue, radiation and immunotherapy induced toxicity can be evaluated.

The goal of this study is to evaluate the safety and mechanisms of action of the novel trimodality treatment approach.

4.2.2 Rationale for Dose Selection/Regimen/Modification

Pembrolizumab will be administered intravenously 200 mg Q3W in this trial. 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.

4.2.3 Translational research

Because the mode of action of combined SBRT and anti-PD-1 therapy is unknown, it is essential to perform translational research to identify the mechanisms of action and toxicity before effect size studies can be initiated with recurrence free and overall survival as outcome parameters.

Serial invasive and noninvasive measurements of immune biomarkers will be performed for the primary tumor, lymph nodes and peripheral blood. A study schedule is provided in section 2.2. In short, the translational research will include:

- Biological imaging of tumor PD-1 expression in patients randomized to combination radiotherapy and pembrolizumab treatment with ⁸⁹Zr-pembrolizumab immuno-PET prior to treatment initiation. Biological imaging of PD-1 expression allows non-invasive *in-vivo* monitoring of PD-1 binding. To visualize PD-1 engagement by pembrolizumab we use a novel technique called immuno-PET in which positron emission tomography (PET) is combined with a radiolabeled monoclonal antibody. Imaging with radiolabelled pembrolizumab (⁸⁹Zr-pembrolizumab) allows for non-invasive quantification of its direct target, the PD-1 receptor on tumor infiltrating lymphocytes. Because the technique is non-invasive and whole body, it allows to evaluate tumor heterogeneity (volumetric analysis of tumor PD-1 expression).
- Immunohistochemical examination of immune effector subsets in a tumor core biopsy specimen at baseline. The following markers will be evaluated for expression: PD-1, PD-L1, CD4, CD8 and FoxP3, combined with Ki67 double staining.
- Multicolor flowcytometric assessment of immune effector subset rates and activation state in peripheral blood and TDLNs by means of endoscopic ultrasound guided fine needle aspiration (EBUS-FNA) at baseline and after SBRT +/- pembrolizumab treatment. The following subsets/populations will be included in this analysis: (memory/effector) CD4+ and CD8+ T cells, Tregs, granulocytic and monocytic MDSCs, conventional and plasmacytoid DCs, and macrophages.
- Three-dimensional tumor and lymph node histological examination of immune effector subsets in the resection specimen after SBRT +/- pembrolizumab treatment. This enables to
 - Explore intratumor heterogeneity of immune effector subsets after SBRT +/- pembrolizumab treatment, possibly associated with therapeutic resistance.
 - Correlate the 3D ⁸⁹Zr-pembrolizumab PET image results with the 3D primary tumor histopathology results and the lymph node histopathology results.
- In the lobectomy specimen the tumor surrounding healthy tissue will be examined for radiation induced changes.

4.2.4 Rationale for Endpoints

This an exploratory study that aims to evaluate the safety of the trimodality approach and characterize the immunological mechanisms of action of combination radiotherapy and immunotherapy. Therefore the primary endpoints of this study are the tolerability and toxicity of combined SBRT +/- pembrolizumab treatment and surgery and to analyze the immunological antitumor response. The endpoints will be assessed by clinical signs of

radiation pneumonitis and examination of tumor core biopsies and resection material, FACS analysis on EBUS-FNA of TDLNs and peripheral blood to detect immunomodulation.

Secondary endpoints are the correlations of *in-vivo* PD-1 expression, quantified by immune-PET, with tissue and blood based immune-parameters.

The levels of PD-1 and PD-L1 expression are variable over time (see section 4.1.8) and expression has been found to be heterogeneous between and within tumor lesions of the same patient. The biomarker PD-L1 IHC is currently being validated in multiple clinical studies to stratify patients based on the intensity and percentage of positive staining. This trial is a unique opportunity to study the 3-dimensional uptake of ⁸⁹Zr-pembrolizumab in the tumor and correlate the imaging result with the 3D immunohistochemistry results of the surgical tumor specimen.

Although pneumonitis will be assessed as a symptom, an exploratory objective is to evaluate radiation induced changes to healthy lung tissue after SBRT +/- pembrolizumab treatment in the resected lung lobe.

The number of patients (n=10 in each arm) is too low to correlate study parameters to clinical outcome in terms of progression-free or overall survival.

4.2.5 ⁸⁹Zr-pembrolizumab imaging

To identify patients that will benefit the most from treatment with mAbs like pembrolizumab, better knowledge of the *in vivo* behavior of the drug is warranted. For this, positron emission tomography (PET) imaging with radiolabeled mAbs (immuno-PET) is an attractive option.

The ability of PET to quantitatively image the distribution of radiolabeled drugs within the body makes this technique a valuable tool for drug development and application. Immuno-PET allows to learn about the ideal drug dosing for optimal tumor targeting (e.g., saturation of receptors), the uptake in critical normal organs to anticipate toxicity, and the variation in pharmacokinetics and tumor targeting. Drug imaging is able to provide this information in an efficient and safe way. Pretreatment imaging with the drug of interest can be used for patient selection because it can show target expression and drug accumulation in tumor lesions and normal tissues noninvasively, quantitatively, and over time. This information might be particularly relevant for heterogeneous target expression or when targeted drugs are combined with other treatment modalities like parallel pathway inhibition, to find routes of synergism. In this way, immuno-PET can be used for better understanding of *in vivo* biology (“immunohistochemistry *in vivo*”) (50).

To enable visualization of a targeted drug with a PET camera, the drug should be labeled with a positron emitter in an inert way, i.e., that neither binding nor pharmacokinetic characteristics of the drug become altered. Moreover, the physical half-life of the positron emitter should be compatible with the residence time of the targeted drug in the body, which is typically several days for slow kinetic intact mAbs. Universal procedures exist for radiolabeling of intact mAbs with the long-lived positron emitter Zirconium-89 (⁸⁹Zr, t_{1/2} = 78.4 h) (51).

During the past years, several preclinical and clinical ⁸⁹Zr-immuno-PET studies have been performed with intact mAbs, for example with mAbs against cMET, EGFR, VEGF and HER2 (52-55).

The first clinical ⁸⁹Zr-immuno-PET study ever was reported in 2006 by Börjesson et al. (56). This feasibility study with 20 head and neck cancer patients showed that ⁸⁹Zr-cmAb, directed

against CD44v6, can be safely applied in patients and that it is a promising tool for PET detection of primary tumors as well as of metastases in the neck. There was good agreement between tumor uptake derived by PET and that derived by tumor biopsy. This suggests that patients with high and low mAb uptake can be differentiated, which might be important for the selection of patients with the highest chance of benefit from mAb therapy. High quality images were obtained in an immuno-PET study with ⁸⁹Zr-trastuzumab in breast cancer patients (57). Excellent visualization of mAb uptake in HER2-positive lesions as well as in metastatic liver, lung, bone, and even brain HER2-positive lesions was observed. ⁸⁹Zr-trastuzumab PET allowed the quantification of conjugate uptake in HER2-positive lesions, and it became clear that for some patients with extensive tumor load, no HER2 saturation occurred during trastuzumab therapy. There was a good correlation between ⁸⁹Zr-trastuzumab tumor uptake and response as assessed by CT, MRI, and bone scans.

Biological imaging of the PD-1 pathway with ⁸⁹Zr-pembrolizumab allows to visualize the PD-1/PD-L1 pathway and non-invasive quantification of its direct target, the PD-1 receptor on tumor infiltrating lymphocytes. Because the technique is non-invasive and whole body, it allows for serial measurements of tumor uptake as well as looking at heterogeneity within and between tumor lesions.

We hypothesize that ⁸⁹Zr-pembrolizumab tumor uptake is heterogeneous within the tumor of individual patients and between tumor lesions of different patients and that uptake is related to PD-1 expression, PD-L1 expression. When a good correlation is found, ⁸⁹Zr-pembrolizumab PET might be used in other clinical settings to validate PD-1 and PD-L1 IHC as predictive biomarker of treatment benefit and to elucidate why some patients with positive PD-L1 IHC do not achieve a favorable tumor response and some patients with negative PD-L1 IHC do.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Patients with early stage (T1bN0 and T2aN0) peripherally located lesions that are cytologically or histologically proven and eligible for surgical resection. Although varying definitions for centrally located tumors have been used, the International Association for the Study of Lung Cancer has recommended the following: a tumor within 2 cm in all directions of any mediastinal critical structure, including the bronchial tree, esophagus, heart, brachial plexus, major vessels, spinal cord, phrenic nerve, and recurrent laryngeal nerve (58).

5.1.2 Subject Inclusion Criteria

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

1. Have a histologically or cytologically confirmed diagnosis of early stage (T1bN0 and T2aN0) peripherally located NCSLC, eligible for surgical resection.
2. Be willing and able to provide written informed consent/assent for the trial.
3. Be ≥ 18 years of age on day of signing informed consent.
4. Have measurable disease based on RECIST 1.1.

5. Must provide tissue from a core or excisional biopsy of the primary tumor lesion.
6. Have a performance status of 0-1 on the ECOG Performance Scale.
7. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 10 days of 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
Renal	
Serum creatinine OR Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) OR ≥45 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

8. 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.
9. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.
10. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

5.1.3 Subject Exclusion Criteria

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

1. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.
2. 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.
3. Has had a prior monoclonal antibody 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.

4. 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., \leq Grade 1 or at baseline) from adverse events due to a previously administered agent.
 - Note: Subjects with \leq 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.
5. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.
6. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.
7. Has a history of (non-infectious) pneumonitis that required steroids, evidence of interstitial lung disease or active, non-infectious pneumonitis.
8. Has an active infection requiring systemic therapy.
9. 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.
10. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
11. 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.
12. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).
13. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
14. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
15. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

5.2 Sample size

Twenty patients will be included in this study. A formal power analysis to calculate a sample size is not feasible due to the explorative nature of this trial. The sample size of 10 patients in each arm is based on two previous analyses that we performed. In a cohort of 9 head and neck cancer patients we were able to find significant differences in the expression of activation markers between different dendritic cell subsets (data on file). In a cohort of 9

melanoma patients we were able to find significant differences in the expression of activation markers between dendritic cell subsets of patients with a Breslow thickness more or less than 1.5 mm (60).

5.3 Pretreatment imaging

5.3.1 Prestudy diagnostic and staging imaging

Standard of care pre-treatment staging includes a diagnostic CT chest and a whole-body static PET-CT.

5.3.2 ⁸⁹Zr-pembrolizumab PET

5.3.2.1 Radiolabeling of pembrolizumab and quality controls

Using standard procedures for the production of ⁸⁹Zr-labeled monoclonal antibodies, several clinical ⁸⁹Zr-immuno-PET studies have been performed, are ongoing, or will be started shortly. In these ongoing studies safety was confirmed. ⁸⁹Zr is coupled to pembrolizumab via the bifunctional chelator desferal. ⁸⁹Zr will be produced at the VU University Medical Center by Cyclotron BV according to procedures essentially as described before (51). Radiochemical purity of the purified ⁸⁹Zr used for labeling will be according to the specification as described in the same paper. ⁸⁹Zr-mAb conjugates produced in this way were found to be fully stable in vitro and in vivo as shown in several preclinical and clinical studies (61). Using the same procedures, the following conjugates have been produced and administered to patients without any adverse event: ⁸⁹Zr-cMAb U36, ⁸⁹Zr-ibritumomab tiuxetan (ZevalinTM), ⁸⁹Zr-rituximab (RituxanTM), ⁸⁹Zr-trastuzumab (HerceptinTM), ⁸⁹Zr-bevacizumab (AvastinTM) and ⁸⁹Zr-cetuximab (ErbixTM) (56, 62).

Radiolabeling of pembrolizumab with ⁸⁹Zr will be performed according to Standard Operating Procedures (SOPs) within the premises of the Radionuclide Center (RNC) of the Department of Nuclear Medicine & PET research. The whole process will be performed according to state-of-the-art Good Manufacturing Practice (GMP) standards. This production site was recently inspected by the Dutch Health Care Inspectorate and is licensed to manufacture tracers for human use according to the latest EU guidelines. This site has also been audited by the largest EU pharma-companies.

The procedures for radiolabeling of mAbs with ⁸⁹Zr have been validated with respect to the synthesis procedure and the final quality of the prepared conjugates. The radiochemical purity, immunoreactivity and endotoxin content of every batch will be assessed prior to administration to a patient. The radiochemical purity as assessed by ITLC (instant thin layer chromatography) and HPLC (high performance liquid chromatography) should always be >90%, preferably >95%, while the immunoreactive fraction as assessed by cell binding assay, should always exceed 70%. This analysis will be performed according to a procedure essentially as described by Lindmo et al (63). The endotoxin content is determined according to pharmacopeia using an endosafe PTS reader. Sterility of each ⁸⁹Zr-pembrolizumab batch will be assured by performing a media fill immediately after final sterilization of each batch. The expiration time of the conjugates will be defined in validations.

5.3.2.2 Radiation exposure

For the patient the radiation dose of administering 37 MBq of ^{89}Zr -pembrolizumab is expected to be around 18 mSv. Low dose CT scans used for attenuation correction will give an additional dose of 3 mSv per CT. The effective dose is therefore expected to be around 24 mSv for the immune-PET scan. After injection no shielding is required and the patient can immediately go home with instructions.

5.3.2.3 Injection procedure

The injection will take place at the department of Nuclear Medicine & PET research. A tracer dose (2 mg) of ^{89}Zr -pembrolizumab will be injected as a bolus of 20 mL with flushing (10 mL physiologic saline).

During administration of ^{89}Zr -pembrolizumab the patient will be monitored closely and appropriate measurements will be performed whenever judged necessary. In case of an allergic reaction to ^{89}Zr -pembrolizumab the patient will be treated according to the standard clinical protocol. In this situation, the patient will be eliminated from the study.

5.3.2.4 Blood sample analysis

Blood samples (7 mL) will be withdrawn at 10 min post-injection, 72 hours and 144 hours post-injection of ^{89}Zr -pembrolizumab to determine ^{89}Zr -kinetics based on measurements of radioactivity.

5.3.2.5 ^{89}Zr -pembrolizumab PET procedure

All PET scans will be performed on a Philips Ingenuity TF PET/CT scanner.

All patients will undergo two whole body PET scans at $t = 72$ and $t = 144$ hours post-injection. The PET scans will be preceded by a 30 mAs low-dose CT, used for attenuation correction and anatomical localization of the PET-signal. Following CT, a total body PET scan will be acquired. PET scans consist of 10-12 bed positions, depending on the length of the patient, of 5 minutes each. Total acquisition time per scan, including low dose CT will be around 60 minutes.

PET data will be normalized and corrected for randoms, tissue attenuation, decay, scatter and dead time. PET-CT data will be reconstructed with TF-OSEM, resulting in a transaxial spatial resolution of ~ 7 mm in the centre of the field of view. Regions of interest (ROIs) will be defined using the CT images which are co-registered to the PET-CT data. ROIs around the organs which accumulate ^{89}Zr (e.g. liver, lung and other metastatic lesions) will be drawn manually directly on the PET images. All tissue concentrations will be related to the blood concentration (venous samples). The radioactivity concentrations in all regions for all imaging time points will be recorded digitally in a spread sheet.

Quantitative accuracy of ^{89}Zr -PET scans was previously confirmed in our center using phantom studies and comparisons between PET-derived and biopsy tumor radioactivity concentrations (64, 65).

5.3.2.6 Analysis of PET data

Tumor volumes of interest will be delineated using semi-automated in-house developed software. SUV_{max} , SUV_{mean} and SUV_{peak} will be calculated for the tumor and visible lymph nodes (67-69).

5.4 On-treatment imaging

The diagnostic CT chest will be repeated within two weeks before surgical resection.

5.4.1 Quantitative changes in lung density

The aim of these measurements is to determine whether a relationship exists between SBRT dose and radiographic lung injury, as measured by CT density changes (70).

Contours of selected isodose levels (0.5 Gy, 3 Gy, 6 Gy, 12 Gy, 18 Gy, 24 Gy, 36 Gy, and 50 Gy) will be exported from the planning system along with the contours of the lung and internal target volume, and the average-intensity CT dataset. These isodose lines are chosen to provide a wide range of doses and large enough volumes between lines to allow for meaningful density measurements. This results in a total of eight evaluable dose-density regions in the ipsilateral lung. In the contralateral lung, only one dose-density region (receiving ≥ 3 Gy) will be analyzed, because it is uncommon for the contralateral lung to receive >5 Gy. The contralateral lung receiving <3 Gy will be considered unirradiated and as such used to correct for baseline differences between scanners. The regions receiving >50 Gy are not analyzed because these volumes are small and because small errors in registration can place a few millimeters of regressing or stable tumor within that volume, artificially increasing density measurements. After deformation of the follow-up scans, isodoses from the endinspiratory phase of the planning CT scan will then be overlaid on the deformed follow-up scan, and changes in Hounsfield unit (HU) density assessed.

The air-filled fraction (f_{air}) will be calculated from HU density using the formula $f_{\text{air}} = \frac{HU - NCT}{-1000 - NCT}$, where NCT is the CT number in HU (71). f_{air} represents the percentage of lung tissue that contains air, and it decreases with increasing CT density.

5.5 Ultrasound endoscopy guided cytological lymph node aspiration for immunological cell composition (EBUS FACS)

5.5.1 Bronchoscopy and EBUS procedure

EBUS-FNA of the ipsilateral and contralateral hilar and mediastinal lymph nodes will be performed within 2 weeks before and between weeks 2 and 3 after SBRT. Patient preparation and sedation will be performed according to regular institutional practice. Needle aspirations will be performed with a 22-gauge needle and 15 passages of the needle per lymph node. The ipsilateral hilar and mediastinal TDLNs will be sampled.

5.5.2 Lymph node analysis

Cells obtained through needle aspirations will be collected in 7.5ml of culture medium (Iscove's Modified Dulbecco's Medium (IMDM)). Since these aspirates contain small tissue components, the specimen will be enzymatically digested for 45 minutes at 37°C in a sterile glass flask by adding 30ml of IMDM with 0.02% DNase and 0.1% Collagenase (60). The cell suspension will then be filtered through a 100µm sterile filter and the flask is rinsed with 20ml IMDM. Cells will be pelleted by centrifugation (5 minutes, 530xg) and counted using trypan blue staining to identify dead cells. Depending on the cell yield, the following immune subsets will be analyzed by flow cytometry using subset-specifying markers (in order of

interest): 1). CD4, CD8 and T regulatory cells (FoxP3), 2). MDSC, 3). T-cell activation and memory/effector phenotypes, 4). myeloid DC subsets, 5). plasmacytoid DC, 6). NK/NKT cells, 7). $\gamma\delta$ T cells and 8) B cells.

For flow cytometry, surface marker staining will be performed by incubating the cells with specific antibodies for 30 minutes at 4°C in PBS supplemented with 1% BSA and 0.02% NaN₃ (FACS buffer). If erythrocytes are abundant in the specimen, these will be lysed by adding BD lysis buffer after 15 minutes of surface antibody staining, after which cells will be stained for an additional 15 minutes. Cells will be washed in FACS buffer and if no intracellular markers need to be stained (FoxP3), cells are ready for analysis. For intracellular staining of FoxP3 and CTLA-4, cells will be permeabilized and fixated using the eBioscience FoxP3 fix/perm kit according to manufacturer's guidelines: cells are first incubated with perm/fix solution for 30 minutes at 4°C and washed with perm/wash solution before blocking with normal rabbit serum (for FoxP3 stainings) and addition of FoxP3 or CTLA-4 specific antibodies. Antibody staining is performed for 30 minutes at 4°C, after which cells are washed once in perm/wash solution and once in FACS buffer prior to analysis on a flow cytometer.

5.6 Treatment plan

5.6.1 Stereotactic body radiotherapy (SBRT)

SBRT delivery will be performed using techniques that are in accordance with the 2017 guidelines of European Society for Radiotherapy and Oncology (ESTRO)-ACROP. An intensity-modulated radiotherapy technique will be used to deliver a risk-adapted protocol, with the dose and number of fractions dependent on the size and location of the tumor (72, 73). Standard SBRT doses will be used in accordance with the previous ROSEL study in operable patients [reference]

5.6.1.1 Immobilization, Imaging and Registration

Patients will be set-up using reproducible positioning, verified using an on-line protocol. Immobilization may include a custom immobilization device, such as a vac-loc bag. All patients treated on a linear accelerator will undergo 4-D planning CT simulation as described previously (72). A 4-D planning scan will not be mandatory in patients who undergo MR-guided SBRT [reference].

5.6.1.2 Volume Definitions and Prescription

The gross tumor volume (GTV) will be defined as the visible tumor on CT or MR-imaging (after verification on planning CT). Planning Target Volume (PTV) will be generated in accordance with ESTRO-ACROP guidelines, and the margins used will depend on the choice of imaging and delivery techniques used. All relevant organs at risk in the proximity of the PTV will be contoured. Dose fractionation schemes to be used are listed in Table 2.

Table 2. Dose fractionation schemes according to tumor location

Tumor Size and Location	Total Dose (Gy)	Number of fractions	Dose per fraction (Gy)	Frequency
Tumors 3 cm or less surrounded by lung	54	3	18	Every second day

parenchyma				
Abutting chest wall or >3 cm	55	5	11	Every second day

Organ-at-risk constraints will be those used in the protocol for a phase III trial in patients who were fit to undergo surgery (74). Doses are prescribed to the 80% isodose line encompassing the PTV, resulting in a hotspot of 120-140% in the ITV. 95% of the PTV should be encompassed by the prescription dose, and 99% of the PTV should be covered by 90% of the prescription dose.

5.6.1.3 Quality Assurance

In order to ensure patient safety and effective treatment delivery, a robust quality assurance protocol is incorporated. The following requirements must be completed for each patient:

- Prior to treatment, each patient must be discussed at departmental quality assurance (QA) rounds.
- All radiotherapy plans must meet protocol dose criteria for organs at risk and plans must be approved by physics and verified by the treating physician. Dose constraints should not be exceeded without prior discussion with the responsible radiation oncologist in the study team.
- Institutional treatment verification procedures may vary based upon equipment available, but compliance with ESTRO-ACROP guidelines must be ensured. On linear accelerators cone-beam CT will generally be used to verify tumor localization prior to treatment, and, if indicated, verification can also be performed during and/or after treatment. For MR-guided adaptive SBRT, real-time imaging using gated delivery can be performed.

5.6.2 Pembrolizumab Treatment

Patients randomized to the pembrolizumab arm will be treated with 200 mg pembrolizumab Q3W for 2 cycles or until unacceptable side effects, starting on the first day of radiotherapy.

5.6.2.1 Dose Modification

Pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs as per Table 3 below.

Table 3: Dose modification guidelines for drug-related adverse events.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below) ^a	Permanently discontinue

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2 ^b	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity ^c	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

^a For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

^b If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to Table 2 – Infusion Treatment Guidelines for further management details.

^c Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued. Subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. For information on the management of adverse events, see Section 5.7.1.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab should be discontinued from trial treatment.

5.6.2.2 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of

each cycle due to administrative reasons. All trial treatments will be administered on an outpatient basis.

Pembrolizumab will be administered as a 30 minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in Section 5.8.1.2). Effort must be made to target infusion timing to be as close to 30 minutes as possible. However, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

5.6.3 Surgical resection

Surgery will occur 8-10 weeks following SBRT +/- pembrolizumab treatment. Surgery will consist of a lobectomy and may employ either an open approach or a video-assisted thoracoscopic approach. Surgical sampling of ipsilateral hilar and mediastinal lymph nodes at the time of resection is mandatory.

5.7 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.7.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

5.7.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids is allowed.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary. The Exclusion Criteria describe other medications which are prohibited in this trial. There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.8 Rescue Medications & Supportive Care

5.8.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis:**
 - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
 - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
 - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis:**

Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

 - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
 - For **Grade 2 diarrhea/colitis**, administer oral corticosteroids.
 - For **Grade 3 or 4 diarrhea/colitis**, treat with intravenous steroids followed by high dose oral steroids.
 - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or \geq Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
 - For **T1DM or Grade 3-4 Hyperglycemia**

- Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
- Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis:**
 - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
 - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism:**

Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

 - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
 - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
 - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
 - **Grade 3-4** hyperthyroidism
 - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic:**
 - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
 - Treat with IV or oral corticosteroids
 - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
 - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis:**
 - For **Grade 2** events, treat with corticosteroids.
 - For **Grade 3-4** events, treat with systemic corticosteroids.
 - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions:** Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 2 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 5 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
<p><u>Grade 1</u> Mild reaction; infusion interruption not indicated; intervention not indicated</p>	<p>Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.</p>	<p>None</p>
<p><u>Grade 2</u> Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for <=24 hrs</p>	<p>Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics</p> <p>Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose.</p> <p>Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.</p>	<p>Subject may be premedicated 1.5h (\pm 30 minutes) prior to infusion of pembrolizumab (MK-3475) with:</p> <p>Diphenhydramine 50 mg po (or equivalent dose of antihistamine).</p> <p>Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).</p>
<p><u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)</p>	<p>Stop Infusion. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine</p>	<p>No subsequent dosing</p>

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 4: Life-threatening; pressor or ventilatory support indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. Subject is permanently discontinued from further trial treatment administration.	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.9 Diet/Activity/Other Considerations

5.9.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.9.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥ 45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2 – Reporting of Pregnancy and Lactation. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.9.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least

monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the PI and to Merck without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the PI and Merck. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the PI and to Merck and followed as described above and in Section 7.2.2.

5.9.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrolment.

5.10 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the PI if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.3.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression
- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Trial Flow Chart) and Section 7.1.4 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up.

5.11 Subject Replacement Strategy

Subjects that fail to undergo the trimodality treatment will be replaced.

5.12 Beginning and End of the Trial

The overall trial begins when the first subject signs the informed consent form. The trial ends when the last subject completes the last trial visit, discontinues from the trial or is lost to follow-up (i.e. the subject is unable to be contacted by the investigator).

We expect to enroll one patient per month. This means that study can be finished within 2.5 years from the time the first subject signs the Informed Consent Form (ICF) through the final contact.

5.13 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to the protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial period	Screening Phase	Imaging	Induction treatment phase	Surgery	Follow-up
Scheduling Window (Days)	-30 to -1	-14 to 0	From day 1 to day 56 (2 weeks after 2 nd pembrolizumab cycle)	From day 56 to day 70	From surgery to 6 months thereafter
Administrative Procedures					
Informed Consent	X				
Eligibility Criteria	X				
Demographics and Medical History	X				
Prior and Concomitant Medication Review	X				
PET Imaging					
⁸⁹ Zr-pembrolizumab		X ^a			
Clinical Procedures/Assessments					
Review Adverse Events	X		X ^b		
Full Physical Examination	X				
Directed Physical Examination	X		X ^b		X ^c
Vital Signs and Weight	X		X ^b		
ECOG Performance Status	X		X ^b		
12-lead ECG	X				
¹⁸ F-FDG PET-CT		X		X	
Laboratory Procedures/Assessments					
Pregnancy Test	X				
Complete Blood Count (CBC)	X	X ⁱ	X ^b		
Comprehensive Serum Chemistry Panel	X		X ^b		
Urinalysis	X				
T3, FT4 and TSH	X		X ^d		
Treatment					
SBRT			X		
Pembrolizumab			X ^a		
Surgery				X	
Efficacy Measurements					
Tumor Imaging (CT thorax +/- abdomen and MRI brain)		X	X ^e		X ^f
Tumor Tissue and Blood analysis					
Core tumor biopsy	X				
EBUS FACS		X	X ^g		
Surgical resection specimen				X	
Immunophenotyping		X ^h	X ^h	X ^h	X ^h

X^a: only for patients randomized to the SBRT + pembrolizumab arm; X^b: every cycle; every 6 weeks; X^c: if indicated; X^d: after 2nd pembrolizumab cycle; X^e: within 2 weeks of surgery; X^f: following resection every 3 months for the first 2 years and every 6 months for the 3 years thereafter; X^g:; X^g:between week 2 and 3 after SBRT; . X^h: see section 7.1.2.10 for timepoints; Xⁱ: 72 and 144 hours post-injection for patients undergoing ¹⁸F-FGD-PET-CT

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements and applicable laws and regulations.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial.

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy.

7.1.1.6 Assignment of Screening Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to the PET scans and pembrolizumab treatment. The screening assignment will start with the term PembroRTSurgery followed by the number of inclusion (e.g. PembroRTSurgery001).

7.1.1.7 Randomization

Patients will be randomized 1:1 by an automated program.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

All AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (irAE). See Section 5.8.1 regarding the identification, evaluation and management of AEs of a potential immunological etiology.

Detailed information regarding the assessment and recording of AEs can be found in section 7.2.

7.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history.

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

Besides the baseline CT, ¹⁸F-FDG PET and ⁸⁹Zr-pembrolizumab PET imaging (described in section 5.3), the diagnostic CT chest will be repeated within two weeks before surgical resection (described in section 5.4). Follow-up following resection will be done with a diagnostic CT chest every 3 months for the first 2 years and every 6 months for the 3 years thereafter, according to institutional standards.

7.1.2.7 Tumor Tissue Collection

7.1.2.7.1 Pretreatment tumor biopsy

Tumor tissue for biomarker analysis from a newly obtained formalin fixed biopsy of the tumor must be provided in the form of a tissue block or at least ten unstained slides. A fine needle aspirate or cytologic specimen will not be acceptable. Needle or excisional biopsies are required. IHC analyses will include PD-1, PD-L1, CD4, CD8 and FoxP3, combined with Ki67 double staining to ascertain effector T cell activation and proliferation in the tumor microenvironment as reported by Tumeh et al. (Nature 2014), also taking into account the effect of quantitative IHC.

7.1.2.7.2 Post-resection specimen

Primary tumor

After resection, the resection specimen will be marked intraoperatively by the surgeon. To enable PET and pathology correlation, the resected specimen and the PET/CT scan are carefully inspected and tissue samples analyzed from the appropriate area of the specimen, according to institutional protocol (75). Margins of specimens will be inked, formalin fixed and sectioned into approximately 1 cm thick slices. Photographs of serial sections will be made. Subsequently, several tissue blocks will be sampled and embedded in paraffin. This facilitates orientation and localization of anatomical structures corresponding to different parts of the tumor on the PET/CT scans. Histological sections of the formalin fixed paraffin embedded blocks from the resected specimens will be examined blinded for the PET data. An estimation of the visually assessed vital tumor percentage will be given for the whole of the tumor. Multiple slides taken from all tumor segments (approximately 1 cm² each) will be scored by the same IHC panel as for the baseline biopsy, which is then used for comparison with SUV values. The presence of tumor cells that may influence the uptake of FDG will also be determined. All IHC parameters will be scored semi-quantitatively (absent = 0, few = 1, moderate = 2, extensive = 3). Tumor segment areas were evaluated per 1 cm², as this approximates the PET resolution and the dimension of the spherical ROI used to determine SUV_{peak}.

Lymph nodes

Immune cells will be isolated from the hilar and mediastinal lymph nodes in the resection specimen by the use of a cytologic scraping method as previously described (60). In short, lymph nodes will be bisected lengthwise with a surgical scalpel. From one half, 10 scrapes are made with a surgical blade (no. 22). The scrape material will be phenotypically analyzed by flow cytometry as described in section 5.5.2.

Tumor surrounding normal lobar lung tissue

The tumor surrounding non-tumor lobar lung tissue will be examined for radiation induced (fibrotic) changes. The percentage of fibrosis and immune cell infiltrate surrounding the tumor will be assessed semi-quantitatively (absent = 0, few = 1, moderate = 2, extensive = 3) (75).

7.1.2.8 Laboratory Procedures/Assessments

The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject can be found in Table 8.

Table 8 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	4	4	16
	Hematology	4	4	16
Pharmacodynamic	PBMC	40	5	200
	⁸⁹ Zr-pembrolizumab PET	7	2	14
Total				246

7.1.2.9 Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 9. Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

Table 9 Laboratory Tests

Hematology	Chemistry	Other
Hematocrit	Albumin	Free thyroxine (T4)
Hemoglobin	Alkaline phosphatase	Thyroid stimulating hormone (TSH)
Platelet count	Alanine aminotransferase (ALT)	
Total WBC	Aspartate aminotransferase (AST)	Blood for correlative studies
	Lactate dehydrogenase (LDH)	
	Creatinine	
	Calcium	
	Glucose	
	Phosphorus	
	Potassium	
	Sodium	
	Magnesium	
	Total Bilirubin	

7.1.2.10 Immunophenotyping on peripheral blood

To evaluate the pre- and on-treatment immune status, polychromatic flowcytometric analyses of lymphoid and myeloid subsets in peripheral blood will be performed at t = 0, 21 days (immediately prior to 2nd therapeutic dose), just before surgery, one week after surgery and after 6 months. Heparinized blood (40 ml) will be taken at each of these time points and PBMC isolated. The following 8-12 marker panels will be run on a BD LSRFortessa (with appropriate FMO controls): 1) Treg/effector T cell activation panel: CD3, CD4, CD8, CD25, CD127, CD45RA, Ki67, FoxP3, HLA-DR, CTLA-4, PD-1, CD27; 2) Dendritic cell panel:

CD1c/BDCA-1, CD202/BDCA-2, CD141/BDCA-3, M-DC8 (Sulph-LacNac), CD14, CD11c, CD16, CD19, CD40, CD80, PD-L1; 3) Myeloid-derived suppressor cell panel: CD11b, CD14, CD33, CD3/19/56 (Lin), HLA-DR, CD15, CD16, PD-L1. By performing these analyses at the indicated time points, we will gain unique insight in the kinetics of T cell activation following SBRT and PD-1 blockade and pre-, on- and post-treatment immune effector/suppressor subset profiles that may show differences between the two treatment arms, as we previously showed in a trial of combined ipilimumab and Prostate GVAX immunotherapy (76-78).

7.1.2.11 Ultrasound endoscopy guided cytological lymph node aspiration for immunological cell composition (EBUS FACS)

Patients will undergo an EBUS-FNA of the ipsilateral and contralateral hilar and mediastinal lymph nodes within 2 weeks before and between week 2 and 3 after SBRT (see section 5.4).

7.1.3 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events.

7.1.4 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.4.1 Screening

7.1.4.1.1 Screening Period

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

Potential subjects will be evaluated to determine that they fulfill the entry requirements as set forth in Section 5.1. Written consent must be obtained prior to performing any protocol specific procedure. Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame. Screening procedures are to be completed within 30 days prior to the first dose of trial treatment except for the following:

- Laboratory tests are to be performed within 10 days prior to the first dose of trial treatment.
- Tumor imaging must be performed within 14 days prior to the first dose of trial treatment.

Subjects may be rescreened after initially failing to meet the inclusion/exclusion criteria. Results from assessments performed during the initial screening period are acceptable in lieu of repeating a screening test if performed within the specified time frame and the results meet the inclusion/exclusion criteria.

7.1.4.2 Treatment Period

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.4.3 Post-Treatment Visits

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures. Subjects will have post-treatment follow-up for experiencing disease progression, until death, withdrawing consent, becoming lost to follow-up or the start of new anti-neoplastic therapy.

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first.

7.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of (⁸⁹Zr-)pembrolizumab, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Adverse events may occur during the course of the use of (⁸⁹Zr-)pembrolizumab in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal. Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 90 days following cessation of treatment or the initiation of new anti-cancer therapy, whichever is earlier and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥ 5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with (“results from”) the overdose of pembrolizumab, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of pembrolizumab meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology “accidental or intentional overdose without adverse effect.”

All reports of overdose with and without an adverse event must be reported within 24 hours to the principal investigator (PI) and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.2 Reporting of Pregnancy and Lactation

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of pembrolizumab, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the PI and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.3 Immediate Reporting of Adverse Events

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of (^{89}Zr -)pembrolizumab that:

- Results in death;
- Is life threatening;

- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Details regarding the above criteria can be found in Table 6.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to (⁸⁹Zr-)pembrolizumab, must be reported within 24 hours to the PI.

In the event of the occurrence of any clinical AE or abnormal laboratory test value that is serious or medically important during the course of the study or the post-treatment period, irrespective of the treatment received by the subject, the investigator is obliged to immediately inform the PI. The immediate report by the investigator to the PI shall be followed by detailed, written, reports using the SAE report form (for an “initial” SAE or for “follow-up” information on a previous SAE).

All SAEs will be reported by the PI through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the PI has first knowledge of the serious adverse reactions.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to (⁸⁹Zr-)pembrolizumab that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the PI and Merck.

All subjects with serious adverse events must be followed up for outcome.

7.2.3.2 Suspected Unexpected Serious Adverse Reactions (SUSARs)

SUSARs will be reported through the web portal *ToetsingOnline* to the accredited METC that approved the protocol and to EudraVigilance, within 15 days after the PI has first knowledge of the SUSAR. SUSARs within this study and SUSARs of pembrolizumab in other studies will be reported separately to the METC.

7.2.3.3 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the PI and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the PI and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to pembrolizumab, must be reported within 24 hours to the PI and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of pembrolizumab, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose, that is not associated with clinical symptoms or abnormal laboratory results.
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.2.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 10. Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.		
	Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.		
	Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.		
	Grade 4	Life threatening consequences; urgent intervention indicated.		
	Grade 5	Death related to AE		
Seriousness	<p>A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:</p> <p>†Results in death; or</p> <p>†Is life threatening; or places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or</p> <p>†Results in a persistent or significant disability/incapacity (substantial disruption of one’s ability to conduct normal life functions); or</p> <p>†Results in or prolongs an existing inpatient hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or</p> <p>†Is a congenital anomaly/birth defect (in offspring of subject taking the product regardless of time to diagnosis);or</p> <p>Is a new cancer; (that is not a condition of the study) or</p> <p>Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.</p> <p>Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).</p>			
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units			
Action taken	Did the adverse event cause the Merck product to be discontinued?			
Relationship to test drug	<p>Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator’s signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information.</p> <p>The following components are to be used to assess the relationship between the Merck product and the AE; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):</p> <table border="1"> <tr> <td>Exposure</td> <td>Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?</td> </tr> </table>		Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?			

Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	The following components are to be used to assess the relationship between the test drug and the AE: (continued)	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial; or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCTPOSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).	
Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.	
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)	

7.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations. The Sponsor will inform Merck Global Safety in the appropriate timelines for reporting Adverse Events.

The PI will submit, once a year throughout the clinical trial, a safety report to the accredited METC.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study.

a report concerning the safety of the patients, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Safety

Safety of the combination of SBRT and pembrolizumab will be assessed by the percentage of ≥ 3 pneumonitis. When combined SBRT and pembrolizumab treatment results in NCIC-CTC grade ≥ 3 pneumonitis in $\leq 10\%$ of patients, the combination is regarded as safe.

8.2 Analysis of immune effector subset data

Immune effector subset expression, expression rate and activation state will be examined in tumor tissue, TDLNs and peripheral blood.

8.2.1 Tumor tissue

IHC analyses will be done on the pretreatment tumor biopsy and postinduction resection specimen of the primary tumor. The analyses include PD-1, PD-L1, CD4, CD8, FoxP3 and Ki67. Values will be quantified semi-quantitatively and compared within each patient (postinduction vs baseline) and between the treatment arms (SBRT vs SBRT + pembrolizumab) with the use of the Wilcoxon signed ranks test and the Wilcoxon-Mann Whitney test, respectively.

Immune cells will be isolated from the hilar and mediastinal lymph nodes in the resection specimen by the use of a cytologic scraping method as described in section 7.1.2.7.2. Values will be quantified on a linear scale and compared between the treatment arms (SBRT vs SBRT + pembrolizumab) with the use of the independent samples t-test.

The tumor surrounding non-tumor lobar lung tissue will be examined for radiation induced (fibrotic) changes. The percentage of fibrosis and immune cell infiltrate surrounding the tumor will be assessed semi-quantitatively and compared between the treatment arms (SBRT vs SBRT + pembrolizumab) with the use of the Wilcoxon-Mann Whitney test.

8.2.2 Immunophenotyping in peripheral blood

All parameters will be quantified on a linear scale and compared within each patient (baseline vs on-treatment vs post-induction vs post-surgery) and between the treatment arms (SBRT vs SBRT + pembrolizumab) with the use of the paired t-test and the independent samples t-test, respectively.

8.2.3 Immunophenotyping on lymph nodes using EBUS-FACS analysis

Absolute values of the individual immune cells will be calculated for TDLNs and NTDLNs.

The percentage difference between TDLNs, NTDLNs, peripheral blood and immune cells isolates from the resection specimen will be calculated and values will be quantified on a linear scale and compared within each patient (postinduction vs baseline) and between the treatment arms (SBRT vs SBRT + pembrolizumab) with the use of the paired t-test and the independent samples t-test, respectively.

8.3 Analysis of PET data

SUV_{max} , SUV_{mean} and SUV_{peak} will be measured in all tumor lesions, enlarged lymph nodes and liver, kidneys, lungs, spleen and left ventricle of the heart.

8.3.1 Heterogeneity analysis

8.3.1.1 Interpatient heterogeneity

Test-retest variability of PET using modern machines is ~15% (79-81). Therefore, uptake difference between patients (interpatient heterogeneity) is defined as SUV differences of 15% or greater.

8.3.1.2 Inpatient heterogeneity

Tracer uptake differences between lesions of the same patient depend on multiple aspects. These are both biological (e.g. the level of perfusion and target expression), as well as technical (e.g. partial volume and tumor motion effects). True biological differences between lesions that correlate with tumor biology are therefore difficult to define. Therefore, inpatient ^{89}Zr -pembrolizumab uptake differences will be assessed qualitatively.

8.3.1.3 Intratumor heterogeneity

Uptake heterogeneity within a tumor also depends on multiple aspects. It can be the result of biological differences, such as perfusion (the tracer is not able to reach some parts of the tumor), true target expression or tumor necrosis. Tumor motion effects also influence intratumor heterogeneity since PET acquisition is not respiratory gated. Intratumor heterogeneity is defined as the ratio of $SUV_{peak} : SUV_{mean}$. The level will be calculated on a linear scale.

8.4 Analysis of CT data

The pre- and post SBRT +/- pembrolizumab CT scans will be assessed for HU density and f_{air} values as outlined in section 5.4.1. Baseline and post induction treatment values within patients will be assessed by the paired t-test. Comparison of the baseline and posttreatment values between the radiotherapy and radiotherapy + pembrolizumab arms will be assessed per time-point by the independent samples t-test.

8.5 Correlation between PET data and Blood and Tissue markers

The correlation between the continuous variable SUV and the categorical variables tissue PD-1 and PD-L1 IHC will be assessed with the ANOVA test. The correlation between the continuous SUV and blood variables will be assessed with the paired t-test.

8.6 Correlation between organ SUV and irAEs

Organ SUV (higher than background uptake vs. equal to or lower than background uptake) will be correlated to irAE (yes or no) using the Chi Square test.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck as summarized in Table 11.

Table 11. Product Descriptions

Product Name & Potency	Dosage Form
pembrolizumab 50 mg	Lyophilized Powder for Injection
pembrolizumab 100 mg/ 4mL	Solution for Injection

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial. Upon completion or termination of the study, all unused and / or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

10.0 ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and last amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen (WMO)).

10.2 Recruitment and consent

Patients eligible for this pilot study will be informed and asked for participation in our outpatient clinic. The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study. It will be emphasized that participation is voluntarily and consent can be retracted at any time.

The investigator is responsible to see that informed consent is obtained from each patient and to obtain the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study drug.

10.3 Benefits and risks assessment, group relatedness

10.3.1 Benefit the trimodality approach

Pooled analysis of our ROSEL study together with the STARS trial from MD Anderson showed that the estimated overall survival at 3 years with SBRT group compares favorably with that of surgery (2). However, despite the promising results of SBRT and this exciting biological mechanism of *in-vivo* vaccination, overall survival was not different between the two modalities. Senthil et al. showed that 22% of patients relapse within 2 years of SBRT treatment. Of these recurrences, 66% were distant (6). The addition of surgery to SBRT enables patients to profit from the *in-vivo* vaccination mechanism and maximizes local control. Preclinical data shows that the addition of anti-PD-1 mAb might synergize with radiotherapy to control distant micrometastases.

10.3.2 Benefit from ⁸⁹Zr-pembrolizumab PET

This is a pilot imaging study. The imaging results will not be used for treatment decision making. Therefore no benefit can be derived from the imaging part of this study. Visualization of tumor uptake and dosimetry of ⁸⁹Zr-pembrolizumab will provide insight in the mechanism of action of pembrolizumab and we hope this study will lead to improved patient selection in future clinical trials to identify which patients benefit the most from treatment with pembrolizumab. We aim to develop immuno-PET in general to provide individualized therapy with monoclonal antibodies in the future (by selection of responders and non-responders). We hope this will contribute to the overall survival of patients and prevention of unnecessary side effects.

10.3.3 Safety of ⁸⁹Zr-pembrolizumab PET

PET imaging with radiolabeled mAbs is safe as outlined in section 5.2.1.

10.3.4 Safety of pembrolizumab treatment

We refer to the Investigator's Brochure for safety analyses in the preclinical and clinical studies. In summary, pembrolizumab has an excellent safety profile.

10.3.5 Safety of the trimodality approach

Combining SBRT with surgery appears safe. At least 4 small studies have reported on patients who have undergone surgery for salvage in patients who have recurred after SBRT (42-45). Since the results of SBRT seem to be equivalent to those of surgery we believe that the delay to surgical resection is not harmful and might even be beneficial for patients. The addition of pembrolizumab to SBRT might lead to a higher rate of pneumonitis. However, the incidence of pneumonitis depends on the target volume and is rarely seen with treatment of early stage lung cancer. Because only patients with peripheral tumors are eligible for this trial and patients receive a lobectomy according to national guidelines on the treatment for NSCLC, the irradiated lung lobe will be resected and resection margins (i.e. central bronchial and vascular structures) are not involved in the radiation target volume.

10.3.6 Radiation exposure

PET

Use of positron emitting radionuclides means exposure to ionizing radiation. The effective dose administration of 37 MBq of ⁸⁹Zr-pembrolizumab is expected to be around 18 mSv, based on data of ⁸⁹Zr-ibritumomab tiuxetan (effective dose 0.55 ± 0.07 mSv/MBq) and ⁸⁹Zr-MAb-U36 (effective dose 0.53 ± 0.03 mSv/MBq in men, 0.66 ± 0.03 mSv/MBq in women) (64, 82). Low dose CT scans used for attenuation correction will give an additional dose of 3 mSv per CT.

When considering the justification of application, a risk to benefit analysis has to be performed. The 'International Commission on Radiological Protection' (ICRP)-62 provides a model for this [International Commission on Radiological Protection. Radiological protection in biomedical research. ICRP Publication 62. Ann ICRP 1993; 22(3)]. The risk level according to this model is stated as Category II "moderate" (effective dosage greater than 10 mSv (adults)) while the social benefit is regarded as "substantial". After injection, no shielding is required and the patient can go home directly with instructions.

Radiotherapy

Radiotherapy can increase the risk of developing a secondary (radiotherapy induced) malignancy. However, because of the stereotactic technique and the low target volume, this risk is expected to be negligible.

10.3.7 Discomfort during scanning

Many patients will be familiar with the PET-CT or CT-scanner from earlier treatment evaluations. To reduce the amount of anxiety and discomfort caused by scanning as much as possible, patients will be informed about the procedure and staff will be present during scanning. If necessary / requested the patient can be removed from the scanner.

10.3.8 Additional blood collection

The total amount of blood to be drawn over the course of the trial is 246 mL.

10.3.9 Additional EBUS

EBUS is a safe procedure that is performed on a weekly basis in our institution. The baseline EBUS procedure is a regular staging procedure and would also be done outside this study protocol. The second EBUS after SBRT +/- pembrolizumab is not part of regular care and will be performed for the purpose of this study. The EBUS procedures will be done under midazolam or propofol sedation according to national guidelines and no harm is expected from this.

10.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.0 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Data Collection

Data collection will be performed by the PI and/or the subinvestigator. Data collection and analysis will be monitored according to good clinical practice and to the Dutch Personal Data Protection Act (De Wet Bescherming Persoonsgegevens).

Case Report Forms (CRFs) for the recording of all data will be developed by the PI. The forms will be printed on normal paper and the original CRFs will be retained by the investigator. Data should be recorded legibly onto the CRF in permanent ink, ballpoint pen. Corrections should be made legibly and initially and dated by approved personnel, the reasons for significant changes must be provided. Correction fluid or covering labels must not be used. Data collected on the CRF are derived from the protocol.

11.2 Handling and storage of data and documents

The investigators will maintain adequate records, including signed patients informed consent forms and information on adverse events. These documents will be kept in a secured area with limited access. All records will be signed and dated by the investigators. All records will be retained for a period of 15 years following the date the entire clinical investigation is completed, terminated or discontinued. The confidentiality will be guaranteed and patients' identification will be coded. The screening assignment will start with the term PembroRTSurgery followed by the number of inclusion (e.g. PembroRTSurgery001). Patient data will be centralized by the coordinating investigator and kept under strict confidentiality. CT data will be stored at the IMS-server for at least 15 years after the last patient of the study has been evaluated or longer if necessary.

Immuno-PET/CT data will be stored in regular diagnostic databases of the nuclear medicine department for at least 15 years after the last patient of the study has been evaluated or longer if necessary.

11.3 Monitoring plan

Representatives of the VUmc will perform on site monitoring visits to verify that trial conduct at the site is in compliance with the Good Clinical Practice (GCP) and the applicable regulatory requirements. Before the study is initiated all aspects of the study are reviewed with the investigators and the staff.

11.4 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC.

All substantial amendments will be notified to the METC and to the competent authority.

11.5 Start of study

The investigator will inform the METC, when the first patient is included.

11.6 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC 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.

11.7 End of study report

The investigator will notify the accredited METC 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 investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.8 Public disclosure and publication policy

With the exception of personal and confidential medical records, all data generated under the trial shall be property of the investigator.

After the last patient entered the study and the results have been obtained and analysed, a study report will be written. The aim is to report the study findings in international peer-reviewed journals and to present the data at international meetings.

11.9 Trial sponsoring and financing

This is an investigator-initiated study. Financial support for this study was received from MSD.

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