SPONSOR: UZ Leuven

TITLE: **Effect of pembrolizumab (Keytruda®) on biomarkers related to intratumoral immunity, proliferation and apoptosis in early breast cancer.**

EudraCT NUMBER: **2016-004859-77**

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Principal investigator **Prof. Dr. Ann Smeets**

Investigators: **Prof. Dr. Ines Nevelsteen and Prof. Dr. Hans Wildiers**

Signature Principal investigator:

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1.0 TRIAL SUMMARY

Abbreviated Title	Effect of pembrolizumab (Keytruda®) on biomarkers in early breast cancer.
Trial Phase	Retrospective study and phase 0 (window-of-opportunity) study
Clinical Indication	Treatment of patients with early breast cancer
Trial Type	Retrospective cohort study and nonrandomized trial
Type of control	NA
Route of administration	Intravenous administration
Trial Blinding	Open label trial
Treatment Groups	Patients with early breast cancer
Number of trial subjects	200 subjects will be included in the retrospective cohort; 54 in the phase 0 trial
Estimated enrollment period	18 months for the phase 0 trial
Estimated duration of trial	24 months
Duration of Participation	6-7 weeks
Estimated average length of treatment per patient	1 day

2.0 TRIAL DESIGN

2.1 Trial Design :

The study consists of 2 parts: a retrospective study, and a prospective clinical study with pembrolizumab (Keytruda®) (Phase 0).
1/ Retrospective study (S58910):
This is a retrospective analysis to study the expression of PD-L1 in ER/PR negative breast tumors and to correlate this PD-L1 expression with tumor infiltrating lymphocytes (TILs), proliferation, expression of apoptosis and clinical outcome (development of distant metastases).
2/ Phase 0 study (S60100):
This is a Phase 0 single center, open-label, non-randomized study in patients with early ER/PR negative breast cancer or early ER positive breast cancer with expression of TILs on core biopsy. Patients will be treated with one injection of Pembrolizumab (Keytruda®) administered intravenously at 200 mg 10 +/- 4 days before surgery. This phase 0 study will consist of 2 cohorts; cohort A will include patients who are scheduled for upfront surgery. Cohort B will include patients who received neoadjuvant chemotherapy (with anti-Her2 therapy if Her2 positive) and who have clear signs of residual tumor on imaging after finishing neoadjuvant chemotherapy (i.e. on imaging estimated residual tumor size of at least 10 mm). The injection will be given in the oncological outpatient unit. Patients will be monitored carefully for the development of adverse experiences/events. Adverse experiences/events will

be evaluated according to criteria outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Cohort A	**Upfront surgery group**	**Cohort B**	**Neo-adjuvant group**
A1	Triple negative	B1	Triple negative
A2	ER-/PR-/Her2+	B2	ER-/PR-/Her2+
A3	ER+ with TILs on core biopsy	B3	ER+ with TILs on core biopsy

2.2 Trial Diagram phase 0 trial

Trial period	**Screening**	**Administration**	**Surgery**	**Post-op**
Schedulling window (days)	-14 to 0	0	10 +/-4	30+/-7
Administrative procedures				
Informed consent	x			
Inclusion/exclusion criteria	x			
Demographics and medical history	x			
Prior and concomittant medication review	x	x	x	x
Labo processing/assesment (local)				
Pregnancy test	x			
CBC with differentiation	x		x	x
Chemistry	x		x	x
TSH, (T3, FT4)	x			x
Urinalysis	x		x	x
Labo (central)				
Blood sample	x		x	x
Tumor tissue collection				
Newly/archival tissue collection	x*		x	
Clinical procedures/assesments				
Review adverse events	x		x	x
ECG	x			
Physical examination	x	x	x	x
Vital signs, weight, height	x	x	x	x
ECOG performance status	x			
Pembrolizumab administration		x		
Anti-cancer therapy status				x
Survival status				x

* Only necessary for cohort B, but will be collected as well in cohort A for further research requiring fresh material, in case patients of cohort A are willing to provide a new fresh tumor sample (with biopsy).

3.0 OBJECTIVES & HYPOTHESES

3.1 Primary Objective & Hypothesis

Objective: To evaluate whether one administration of pembrolizumab (Keytruda®) is able to alter biomarkers related to intratumoral immunity (i.e. the amount of TILs, PD-L1 and CD73 expression) and proliferation (i.e. Ki67 expression) in early breast cancer

Hypothesis: One administration of pembrolizumab (Keytruda®) will result in an increase in the amount of TILs and a decrease in the expression of PD-L1, CD73 and Ki67 in a subgroup of patients with early breast cancer.

3.2 Secondary Objectives & Hypotheses

PROSPECTIVE STUDY:

(1) **Objective**: To evaluate whether anthracycline-taxane based neo-adjuvant chemotherapy is able to alter biomarkers related to intratumoral immunity and proliferation in early breast cancer.

Hypothesis: Administration of anthracycline-taxane based neo-adjuvant chemotherapy will result in an increase in the amount of TILs and a decrease in the expression of PD-L1, CD73 and Ki67 in a subgroup of patients with early breast cancer.

(2) **Objective**: To evaluate whether there is a difference in biomarker evolution after pembrolizumab (Keytruda®) administration in cohort A (upfront surgery, not prior treatment) or cohort B (have received full course of neoadjuvant chemotherapy, and have clear evidence of residual tumor after chemotherapy).

Hypothesis: The change in biomarker expression will be higher in cohort B (having received full course of neoadjuvant chemotherapy, and having clear evidence of residual tumor after chemotherapy) than in cohort A (upfront surgery, not prior treatment).

(3) **Objective:** To evaluate whether there is a difference in biomarker evolution after one pembrolizumab (Keytruda®) administration in triple negative breast cancer (Cohort A1,B1) versus ER/PR negative Her2 positive breast cancer (Cohort A2,B2).

Hypothesis: The change in biomarker expression after pembrolizumab (Keytruda®) administration will be higher in triple negative breast cancer than in ER/PR negative Her2 positive breast cancer.

(4) **Objective:** To evaluate whether there is a difference in biomarker evolution after one pembrolizumab (Keytruda®) administration in triple negative breast cancer (Cohort A1,B1) versus ER positive breast cancer (Cohort A3,B3).

Hypothesis: The change in biomarker expression after pembrolizumab (Keytruda®) administration will be higher in triple negative breast cancer than in ER positive breast cancer.

(5) **Objective:** To evaluate whether there is a difference in biomarker evolution after one pembrolizumab (Keytruda®) administration in ER/PR negative Her2 positive breast cancer (Cohort A2,B2) versus ER positive breast cancer (Cohort A3,B3).

Hypothesis: The change in biomarker expression after pembrolizumab (Keytruda®) administration will be higher in ER/PR negative Her2 positive breast cancer than in ER positive breast cancer

(4) **Objective:** To evaluate whether one administration of pembrolizumab (Keytruda®) causes a significant shift in T-cell subsets in peripheral blood.

Hypothesis: One administration of pembrolizumab (Keytruda®) causes a significant shift in T-cell subsets in peripheral blood.

(5) **Objective:** To evaluate whether baseline PD-L1 expression and amount of TILs are predictive for the effect of pembrolizumab (Keytruda®) and/or response to neo-adjuvant chemotherapy.

Hypothesis: Tumors with a high baseline PD-L1 expression and a high amount of TILs are more likely to respond to pembrolizumab (Keytruda®) and/or response to neo-adjuvant chemotherapy than tumors with a low baseline PD-L1 expression and/or a low amount of TILs.

RETROSPECTIVE STUDY:

(6) **Objective:** To evaluate whether baseline expression of PD-L1, CD73, Ki67 and the amount of TILs can predict distant metastasis free survival.

Hypothesis: Low expression of PD-L1, CD73 and Ki67 and a high amount of TILs are associated with a better distant metastasis free survival.

(7) **Objective:** To evaluate whether there is an association between expression of PD-L1, CD73, Ki67 and the amount of TILs.

Hypothesis: There is an association between expression of PD-L1, CD73, Ki67 and the amount of TILs.

(8) **Objective:** To evaluate whether the immunohistochemical analysis of PD-L1, CD73, Ki67 and TIL count on core biopsy specimens reflects accurately the expression of these markers on tumor resection specimen.

Hypothesis: The immunohistochemical analysis of PD-L1, CD73, Ki67 and TIL count on core biopsy specimens reflects accurately the expression of these markers on tumor resection specimen.

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Early breast cancer

Breast cancer is the most common invasive malignancy and the most common cause of cancer-related mortality in women. The lifetime probability of developing invasive breast cancer in the United States and Europe is one in eight. Although distinct efforts have been made to optimize breast cancer treatment, up to 20-30% of early breast cancer patients will eventually develop metastases. The risk of developing distant metastases is related to the stage of disease at diagnosis and tumor biology. Hormone receptor (ER/PR) negativity is a major, poor prognostic factor. ER/PR negative breast cancer occurs most frequently in younger women, is associated with a more aggressive behavior and poorer prognosis. Although impressive efforts have been made to optimize treatment modalities in this subtype of breast cancer, the therapeutic options are still rather limited, compared to ER/PR positive breast cancer. Therefore, many researchers are still challenged by the search for new, eligible therapeutic targets.

4.1.2 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ

and ZAP70 which are involved in the CD3 T-cell signaling cascade. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda® has been approved in Belgium for inoperable or metastatic melanoma and non-small lung cancer.

4.1.3 Biomarkers to predict and monitor response to pembrolizumab (Keytruda®)

As only a subset of patients treated with immune checkpoint antibodies experience durable and long-term disease control, predictive biomarker development has become a top priority. Tumor PD-L1 expression has been pursued as a potential biomarker and studies have demonstrated that PD-L1 baseline expression level showed strong association with response to anti-PD-1 therapy. However, clinical responses were observed also in patients considered to be negative for PD-L1 expression in the tumor. However, the appearance of measurable anti-tumor activity may take longer for immune therapies than for cytotoxic therapies and lesions may enlarge before shrinking. Therefore, identification of biomarkers to monitor early response are urgently needed.

4.1.4 Preclinical and Clinical Trial Data

We refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

There is an urgent need for new treatment strategies in patients with ER/PR negative breast cancer. Different clinical studies with anti-PD-1 therapy show clear evidence of clinical benefit in terms of overall survival, demonstrating immune modulating of ICPs as a promising new

targeted therapy in different types of cancer, even in less immune-driven subtypes of cancer. However, most of these studies are performed in metastatic stages of malignant melanoma, non-small cell lung cancer etc. No studies are available on the response to immune therapy in early stages of cancer. In addition, data on ICPs-expression and the effect of ICP- inhibition therapy (e.g. PD-1 inhibition) are rather scarce in breast cancer, as well as criteria to evaluate response in early stage of disease. Investigation of potential biomarkers is urgently needed to identify the subgroup of patients that will benefit from immunotherapy. This will optimize the therapeutic efficacy, prevent toxicity and reduce the costs associated with immunotherapy.

Tumor cells show increased response to immunotherapy after they became more immunogenic by therapy-induced immunogenic cell death (ICD) e.g. chemotherapy, radiotherapy etc. This process might be of special interest/benefit in less immunogenic-driven tumors. BC has traditionally not been considered as a highly immunogenic neoplasm, but there is increasing evidence for dynamic cross-talk between the host-immune system and BC cells. In addition, certain subgroups of BC (mainly ER/PR negative tumors) display presence of a massive lymphocytic tumor-infiltrate (TILs), which have been shown to be a potential key factor in the response to immunomodulatory therapy. Therefore, anthracycline based chemotherapy, followed by immunotherapy (e.g. PD-1 inhibition therapy) might be promising in BC.

There are currently little data regarding efficacy of immunotherapy in ER+ breast cancers. This subtype is generally considered to be less immunogenic than ER/PR negative breast cancer. The relationship in ER+ breast cancer is more complicated due to the association of TILs with higher proliferation rates, with the latter being associated with poorer outcomes in luminal breast cancers. However, immunotherapy could be an option for patients in with ER+ breast cancer with a high amount of TILs.

This phase 0 study allows short-term evaluation of the effect of anti-PD1 therapy in early breast cancer with biomarker determination by comparing histological markers pre-treatment and at surgery.

Changes in biomarkers related to intratumoral immunity and proliferation may provide evidence for biological acitivity of pembrolizumab (Keytruda®) and may allow the development of a blood-based biomarker to help predict which patients may benefit form Pembrolizumab (Keytruda®) and to monitor therapy response.

To evaluate whether neo-adjuvant chemotherapy can alter expression of biomarkers related to intratumoral immunity and proliferation, patients will also be enquired to provide a new core biopsy after the last dose of chemotherapy.

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label Phase 1 trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in

subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

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

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

4.2.3 Rationale for Endpoints

4.2.3.1 Biomarker Research

PD-L1: Previous studies have shown a correlation between PD-L1 expression and an objective response in patients treated with anti-PD-1/L1 therapy. However this finding is not consistent among all studies. It also raises the question whether clinical decisions regarding the treatment of patients who have failed conventional therapies and for whom no other treatments are available should be based on PD-L1 expression. On the basis of these controversial results, we will evaluate the potential predictive role of PD-L1 expression in tumor cells.

TILs: TILs might be the primary predictors of response to ICP therapy. Tumors with a large amount of TILs are more likely to respond to anti PD-1/L1 therapy.

Ki67: Immunohistochemical assessment of the proportion of cells staining for the nuclear antigen Ki67 has become the most widely used method for comparing proliferation between tumor samples. In spite of consistent data on Ki67 as a prognostic marker in early breast cancer, its role in breast cancer management remains uncertain. Reductions in Ki67 occur in the tumors of most patients receiving systemic treatment, and there is some evidence that there are greater reductions in patients who respond to treatment.

Except from the above mentioned biomarkers will blood and tissue samples be used to study other possible biomarkers and T-cell subpopulations. These tests will be performed in cooperation with KU Leuven laboratories.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

For the retrospective study (S58910)

All breast cancer patients diagnosed and treated at the Leuven Multidisciplinary Breast Center (University Hospitals Leuven) are documented in a clinical-pathological database, containing extensive general and tumor-related information, as well as clinical follow-up such as relapse and cause of death. This database contains data from over 10000 patients and is linked to a tumor and blood bank. For the proposed study patients will be selected from this database based on the following criteria:

- Diagnosed between 2005 and 2010 with non-metastatic primary invasive breast cancer
- Primary surgery in our institution
- Treated with upfront surgery
- ER/PR negative breast cancer (Allred score)
- Primary tumor >2cm
- Stratification for Her2 status evaluated using IHC followed by FISH

For the prospective Phase 0 study

Patients must meet all of the following criteria to be eligible for study entry:

- Non-metastatic newly diagnosed primary invasive carcinoma of the breast
- Primary tumor > 1 cm
- Negative estrogen (ER) and progesterone (PR) receptor status OR
  Positive estrogen receptor (ER) status with TIL expression on core biopsy

5.1.2 Subject Inclusion Criteria

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

1. Be willing and able to provide written informed consent/assent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion if needed. *Newly obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 0. Subjects for whom newly obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Sponsor.*

4. Have a performance status of 0 or 1 on the ECOG Performance Scale.

5. Have non-metastatic operable newly diagnosed primary invasive carcinoma of the breast that is:

    a. Histologically confirmed

    b. ER/PR negativeor ER positive breast cancer with TIL expression on core biopsy. ER/PR status will be evaluated with Allred score (semi-quantitative measurement) following ASCO CAP guidelines 2009. TIL count based on the guidelines of the International TILS Working Group on Breast Cancer

    c. HER2 negative of positive. HER2 status will be evaluated using IHC followed by FISH with dual probe (ASCO CAP guidelines 2013).

d. Primary tumor size greater than 1 cm, measured by any of clinical examination, mammography, ultrasound or magnetic resonance imaging

e. Any clinical nodal status

6. Have evaluable core biopsy for IHC

7. Be willing to provide plasma/blood samples

8. After neo-adjuvant chemotherapy (cohort B1 and B2) patients must have residual tumor >1cm and must be willing to provide evaluable new tumor biopsy for IHC

9. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 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 without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

10. Female subject of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving the study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

11. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after receiving the study medication.

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

12. Male subjects of childbearing potential (Section 5.7.1) must agree to use an adequate method of contraception as outlined in Section 5.7.1- Contraception, until 120 after receiving the study therapy.

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

5.1.3 Subject Exclusion Criteria

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

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of receiving the treatment dose.

2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to receiving the trial treatment.

3. Has a known history of active TB (Bacillus Tuberculosis)

4. Hypersensitivity to pembrolizumab or any of its excipients.

5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks (or except for Trastuzumab within 2 weeks) prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 0 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

7. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

8. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive

drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

9. Has known history of (non-infectious) pneumonitis that required steroids or current pneumonitis.

10. Has an active infection requiring systemic therapy.

11. 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.

12. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

13. 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.

14. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2 agent or co-inhibitory T-cell receptor therapy (e.g. OX40-CD137, CTLA-4)

15. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

16. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

17. Has received a live vaccine within 30 days of planned start of study therapy.

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

5.2 Trial Treatments

5.2.1 Study cohorts prospective trial

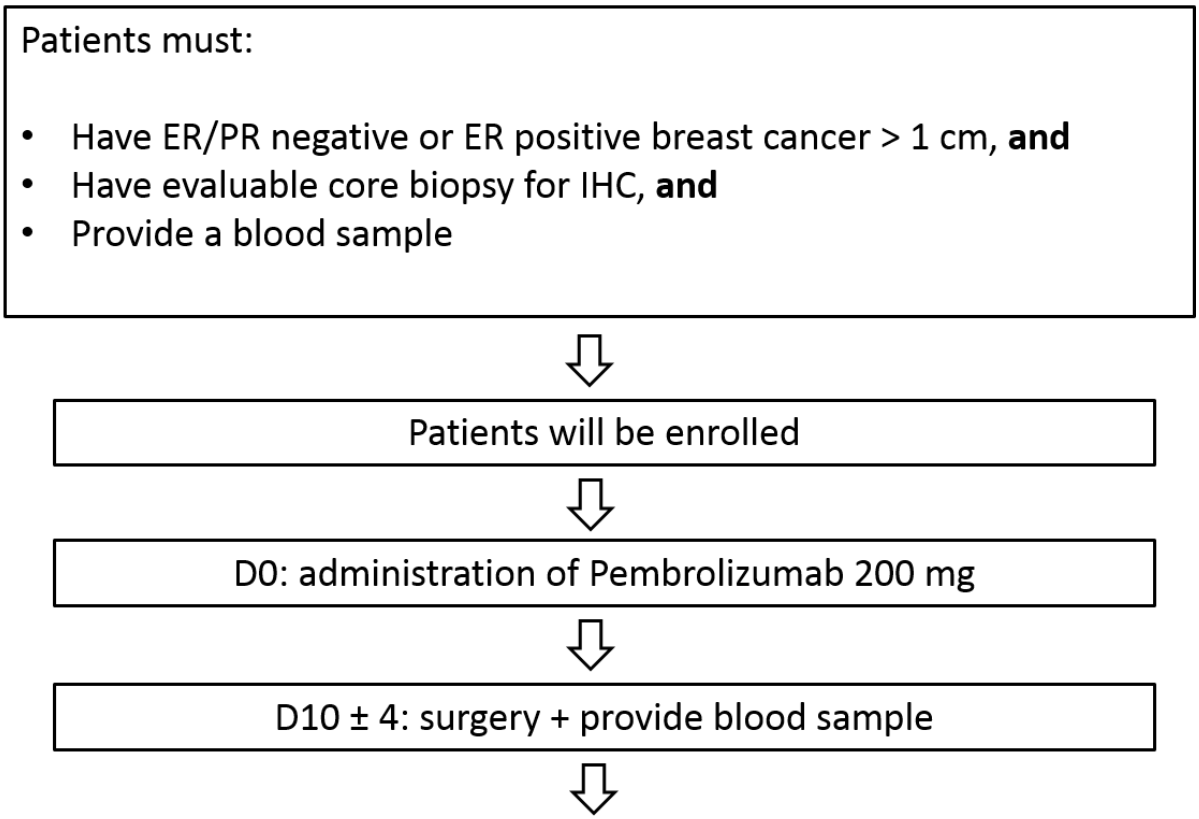
- Cohort A1: upfront surgery, triple negative
- Cohort A2: upfront surgery, ER/PR negative, Her2 positive
- Cohort A3: upfront surgery, ER positive with TILs on corebiopsy
- Cohort B1: tumor >1cm after neo-adjuvant anthracycline-taxane based chemotherapy, triple negative
- Cohort B2: tumor >1cm after neo-adjuvant anthracycline-taxane based chemotherapy, ER/PR negative, Her2 positive
  Cohort B3: tumor >1cm after neo-adjuvant anthracycline-taxane based chemotherapy, ER positive with TILs on corebiopsy

5.2.2 Study cohorts prospective trial

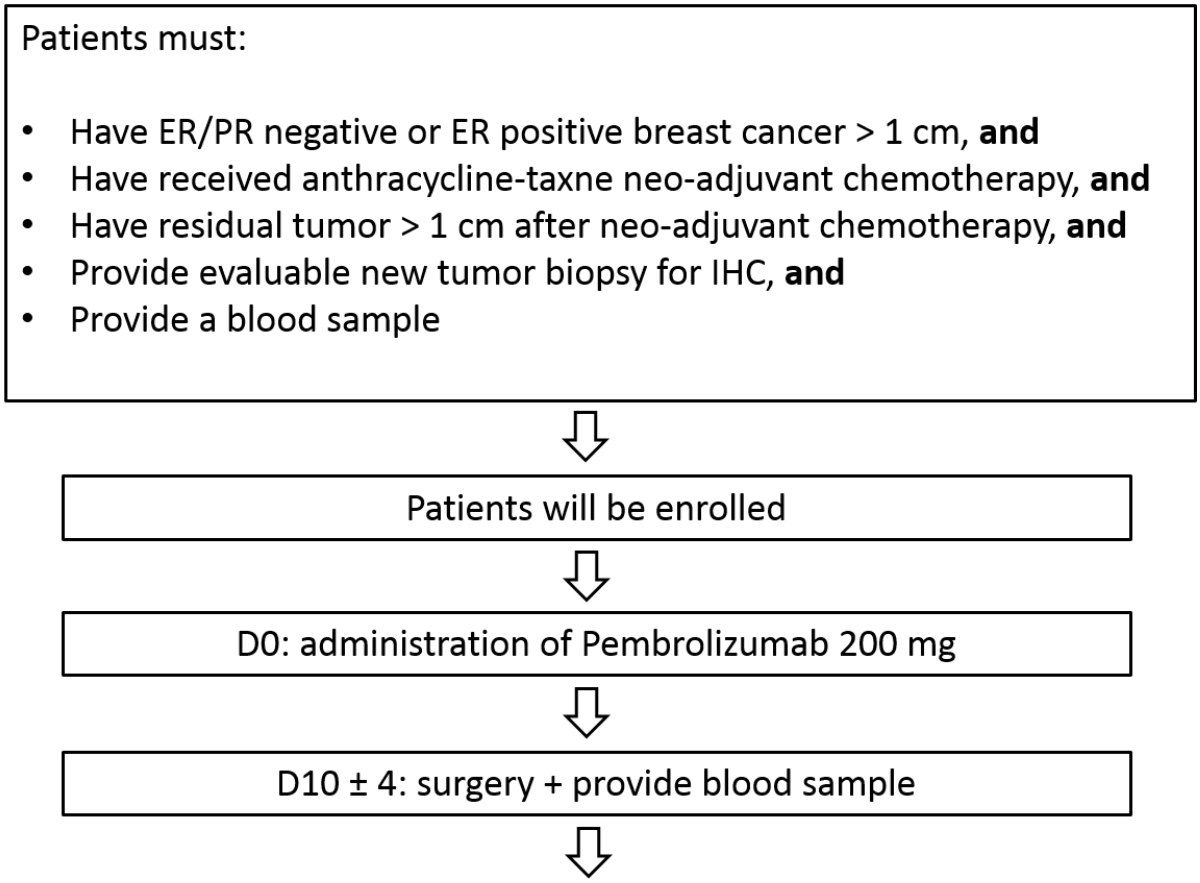
The trial design for each cohort is displayed below.

All patients will receive 1 administration of pembrolizumab 10 +/- 4 days before surgery

Cohort A



Cohort B



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

New tumor biopsy only necessary for cohort B, but will be collected as well in cohort A for further research requiring fresh material, in case patients of cohort A are willing to provide a new fresh tumor sample (with biopsy).

5.2.3 Dose Selection

5.2.3.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

5.2.4 Timing of Dose Administration

Trial treatment should be administered after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0).

All trial treatments will be administered in day hospital setting.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion 10+/-4 days before surgery. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.2.5 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 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 investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

5.3.1 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. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before receiving the trial treatment and 30 days after receiving the trial treatment should be recorded. Concomitant medications administered after 30 days after receiving the trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.3.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy

- Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.

- 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, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- Systemic 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 may be approved after consultation with the Sponsor.

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 describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.4 Rescue Medications & Supportive Care

5.4.1 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 ≥ 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 liothyroinine, 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 2 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	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. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 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) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine 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.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

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

5.5.2 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.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level

in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

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 subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 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 Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck 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 Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.5.4 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 enrollment.

5.6 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 Sponsor 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 7.1.4 – Other Procedures.

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.
- Unacceptable adverse experiences as described in Section 5.2.1.2
- 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 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.2.3.1). 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. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.7 Subject Replacement Strategy

A patient who discontinues the trial will not be replaced.

5.8 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 protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 TRIAL FLOW CHART

Study Flow Chart

Trial period	Screening	Administration	Surgery	Post-op
Schedulling window (days)	-14 to 0	0	10 +/-4	30+/-7
Administrative procedures				
Informed consent	x			
Inclusion/exclusion criteria	x			
Demographics and medical history	x			
Prior and concomittant medication review	x	x	x	x
Labo processing/assesment (local)				
Pregnancy test	x			
CBC with differentiation	x		x	x
Chemistry	x		x	x
TSH, (T3, FT4)	x			x
Urinalysis	x		x	x
Labo (central)				
Blood sample	x		x	x
Tumor tissue collection				
Newly/archival tissue collection	x*		x	
Clinical procedures/assesments				
Review adverse events	x		x	x
ECG	x			
Physical examination	x	x	x	x
Vital signs, weight, height	x	x	x	x
ECOG performance status	x			
Pembrolizumab administration		x		
Anti-cancer therapy status				x
Survival status				x

* Only necessary for cohort B, but will be collected as well in cohort A for further research requiring fresh material, in case patients of cohort A are willing to provide a new fresh tumor sample (with biopsy).

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 by the Sponsor and/or Merck 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.

In obtaining and documenting informed consent, the investigator should comply with applicable regulatory requirements and should adhere to good clinical practice (GCP) guidelines and to the ethical principles that have their origin in the Declaration of Helsinki.

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. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

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 7 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

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

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. 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. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.6 Assignment of Screening Number

Not applicable

7.1.1.7 Assignment of Randomization Number

Not applicable

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 and during the follow-up period according to NCI CTCAE Version 4.03 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

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. A full physical exam should be performed during screening,

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 (see Section 11.1) 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

According to the NCCN guidelines version 2.2016 a bilateral mammogram and ultrasound of the breast and regional lymph nodes will be performed at diagnosis. All patients will undergo a core needle biopsy of the tumor and determination of tumor ER/PR status (Allred score) and Her2 status (ICH followed by FISH). A fine needle aspiration cytology (FNAC) will be performed of suspicious axillary lymph nodes. Breast MRI will be performed when indicated.

Bone scan, chest X-rays/diagnostic computed tomography (CT), magnetic resonance imaging (MRI), liver imaging, 18-fluoro-deoxyglucose positron emission tomography (PET) scans, and/or other radiographic modalities may be performed as clinically indicated according to NCCN or national guidelines. A baseline chest X-ray will be obtained. These radiographic assessments may be considered when clinically indicated to exclude metastatic disease at baseline or assess distant disease recurrence status during study treatment.

Distant recurrence
Defined as evidence of tumor in all areas, with the exception of loco regional areas.
Confirmed by the following criteria:

-Skin, subcutaneous tissue, and lymph nodes (other than local or regional)
Positive cytology, aspirate, or biopsy OR radiologic (CT scan, MRI, PET, or ultrasound) evidence of metastatic disease

-Bone
X-ray, CT scan, or MRI evidence of lytic or blastic lesions consistent with bone metastasis, OR bone scan (requires additional radiologic investigation; alone not acceptable in case of diagnostic doubt) OR biopsy proof of bone metastases or cytology

-Bone marrow
Positive cytology or histology or MRI scan

-Lung
Radiologic evidence of multiple pulmonary nodules consistent with pulmonary metastases
Positive cytology or histology (in practice, rarely performed except in the case of solitary nodules)
Note: For solitary lung lesions, cytologic or histologic confirmation should be obtained in case of diagnostic doubt. Proof of neoplastic pleural effusions should be established by cytology or pleural biopsy.

-Liver
Radiologic evidence consistent with liver metastases OR liver biopsy or fine needle aspiration
Note: If radiologic findings are not definitive (especially in the case of solitary liver nodules), a liver biopsy is recommended; however, if a biopsy is not performed, serial scans should be obtained, if possible, to document stability or progression.

-Central nervous system
Positive MRI or CT scan, usually in a patient with neurologic symptoms, OR biopsy or cytology (e.g., for a diagnosis of meningeal involvement). However, meningeal involvement may also be

diagnosed by CT scan or MRI and, depending on the general status of the patient, additional investigations (including cytology of the cerebrospinal fluid).

The investigator or qualified designee will review all technical examinations related to the disease.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Alkaline phosphatase	Test strip screening:	Serum β-human chorionic gonadotropin†
Hemoglobin	Alanine aminotransferase (ALT)	Glucose	(β-hCG)†
Platelet count	Aspartate aminotransferase (AST)	Ketons	PT (INR)
WBC (total and differential)	Lactate dehydrogenase (LDH)	Specific gravity	aPTT
Red Blood Cell Count	Biocarbonate	Heam	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Ureum	pH	Free tyroxine (T4)
Absolute Lymphocyte Count	Total Calcium	Protein	Thyroid stimulating hormone (TSH)
	Creatinine	Nitrite	
	Chloride	Leuco-esterase	
	Phosphate	Bilirubine	Blood for correlative studies
	Potassium	Urobilinogeen	
	Sodium		
	Gamma GT	Urine pregnancy test †	
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in your region.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 14 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.

7.1.4 Other Procedures

7.1.4.1 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. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.5.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.4.2 Blinding/Unblinding

Not applicable

7.1.5 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.

7.1.5.1 Screening

7.1.5.1.1 Patients with ER/PR negative OR ER positive with TILs on core biopsy, and residual tumour of at least 1 cm after neo-adjuvant chemotherapy will be screened before surgery as well as patients with ER/PR negative breast cancer only scheduled for surgery and adjuvant therapy if applicable. Screening Period

Patients will be screened at a maximum of 14 days before administation of Pembrolizumab.

7.1.5.2 Treatment Period

Patients in the phase 0 study will be treated with one injection of Pembrolizumab (Keytruda®) administered intravenously at 200 mg 10 +/- 4 days before surgery.

7.1.5.3 Post-Treatment Visits

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. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.1.5.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.5.4 Follow-up Visits

Not applicable

7.1.5.5 Second Course Phase (Retreatment Period)

Not applicable

7.2 Assessing and Recording Adverse Events

According to the ICH guideline for Good Clinical Practice, 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 the Merck's product, 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.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All adverse events 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.

From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded 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. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

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 a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product 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 Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

7.2.2 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

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 Sponsor's product, 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 Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

7.2.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.2.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product 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 another important medical event

- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
    - Is a new cancer (that is not a condition of the study);
    - Is associated with an overdose.

Refer to Table 6 for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause that occurs to any subject must be reported within 24 hours to the Sponsor 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 serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

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

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

7.2.3.2 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 Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

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 Sponsor 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 Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, 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.

7.2.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting.

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 7.2.3.- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g.transportation issues etc.) will not be considered a SAE.

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 6 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 mid 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	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**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	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**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 for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. 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 to the Sponsor and to Merck within 2 working days..	

	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 †).	
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 Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that 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. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to 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 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 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 Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to 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) Sponsor's 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 MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR 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 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 Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.
No, there is not a reasonable possibility of Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (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.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Determination of sample size

Retrospective study (S58910):

Between 2005 and 2010, 285 patients with a ER/PR negative breast cancer larger than 2cm were diagnosed and treated with upfront surgery at the Leuven Multidisciplinary Breast Center (University Hospitals Leuven). Two hundred consecutive patients with available tumor tissue and follow-up data will be included in the proposed retrospective study.

Prospective study (S60100):

The assumptions for the sample size are as follows: More than 600 patients with early breast cancers are diagnosed and treated at the Leuven Multidisciplinary Breast Center (University Hospitals Leuven) on a yearly basis. Twenty percent (120 patients) of these breast cancers are ER/PR negative, half of them Her2 negative and half of them Her2 positive. Of the 120 ER/PR negative patients, 25 percent (30 patients) is expected to receive upfront surgery and 75 percent (90 patients) upfront chemotherapy. After upfront chemotherapy 45 percent of patients is expected to have a residual disease larger than 1cm (40 patients). In total, 70 patients are expected to be eligible for the suggested prospective study. Participation of 50 percent of eligible patients would allow inclusion of 34 patients after 1 year.

Approximately 66 percent (400 patients) of all patients treated at the Leuven Multidisciplinary Breast Center have ER positive breast cancer. 10 percent of these ER+ positive patients is expected to be eligible for this trial taking into account the tumour size after neo-adjuvant therapy and a decent expression of TILs on core biopsy. Participation of 50% of eligible patients would allow inclusion of 20 patients after 1 year. Patients will be included until we have reached the total of 54 patients, independent of the amount of patients in the different cohorts

8.2 Statistical analysis plan

Retrospective study:

Pearson correlations will be used to analyze the association between expression of PD-L1, CD73, Ki67, the amount of TILs, and other relevant functional immunoparameters. Linear regression analysis will be applied to estimate the predictive value of the core needle biopsy expression measurements of PD-L1, CD73, Ki67 and TIL count for the expression of the same parameters in the whole tumor as measured by the surgical resection specimens.

Prospective study:

Changes in biomarker levels within patients between measurements before and after the administration of pembrolizumab will be analyzed using paired t-test or Wilcoxon signed rank test, as appropriate depending on the distribution of the measurements.

Differences in evolutions of biomarker expression between cohorts will be estimated using linear models and presented as the mean difference of the change with 95% confidence intervals.

Statistical analysis will be performed by an experienced biomedical statistician at the Interuniversity Centre for Biostatistics and Statistical Bioinformatics.

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 7.

Table 7 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 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.
Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.
Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.
Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA and other national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC, as appropriate.

10.2 Compliance with Financial Disclosure Requirements

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (i.e., last patient, last visit).

10.3 Compliance with Law, Audit and Debarment

This study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

10.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.5 Quality Management System

Specimens will be tracked in a manner consistent with Good Clinical Practice by a quality-controlled, auditable, and appropriately validated laboratory information management system, to ensure compliance with data confidentiality and adherence to authorized use of specimens as specified in this protocol and in the Informed Consent Form. The site will permit monitoring, audits, IRB/EC review, and health authority inspections by providing direct access to source data and documents related to the samples.

10.6 Data Management

10.6.1 Investigator's file

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories: Investigator's study file and patient clinical source documents.

The investigator's study file should be established according to the ICH-GCP E6 and the list of essential documents to be collected and kept up-to-date in this file will be provided to the investigator; Subject clinical source documents would include subject hospital/clinic records; physician's and nurse's notes; appointment book; original laboratory reports; signed informed consent form and consultant letters.

10.6.2 Case Report Form (CRF)

The CRF will be either in a paper version or in an electronic version (eCRF). In case of an eCRF, it will be completed electronically with a personal access code. An audit trail will maintain a record of the initial entries and changes made, reasons for change, time and date of entry, and user name of person authorizing entry or change. In case of a paper CRF, all form should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initiated and dated. For each subject enrolled, a CRF must be completed by the investigator or authorized delegate from the study staff. The Investigator is responsible to make a delegation log mentioning responsible persons to complete and sign the CRF. The investigator should ensure the accuracy, completeness, legibility and timeliness of the data reported in the CRFs and in all required reports. Data reported in the CRF must be derived from source documents and should be consistent with the source documents. Any change or correction to a CRF should be dated, initialed and explained (if necessary). The Investigator will retain a copy of the printed and signed CRF. The Investigator will also retain copies of any data query forms (e.g. lab tests). For all patients the monitor will check the CRFs.

11.0 APPENDICES

11.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

11.2 Common Terminology Criteria for Adverse Events V4.03 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for adverse event reporting. (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf)

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

In addition, volumetric analysis will be explored by central review for response assessment.