Protocol GEICAM/2015-04
"A multicenter phase II trial to evaluate the efficacy and safety of pembrolizumab and gemcitabine in patients with HER2-negative Advanced Breast Cancer (ABC)"
"PANGEA-Breast"

SPONSOR:

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SUMMARY OF THE STUDY PROTOCOL

Study Title: A multicenter phase II trial to evaluate the efficacy and safety of pembrolizumab and gemcitabine in patients with HER2-negative Advanced Breast Cancer (ABC) "PANGEA-Breast"

Sponsor Study Code: GEICAM/2015-04

Sponsor: GEICAM (Spanish Breast Cancer Research Group Foundation)

Indication: HER2-negative Advanced Breast Cancer (ABC)

Countries and approximate number of sites: Spain / 10 sites

Number of patients: 53 patients (up to a maximum of 65 patients if 12 additional patients are needed due to the run-in-phase).

Study Rationale:

Available data support the hypothesis of an immune mediated antitumor activity in breast carcinoma, and several lines of research are ongoing. It is critical to understand what happens in the tumoral microenvironment in order to design biological agents and approaches that might modulate the immune response towards cancer cell destruction. At this point and due to new knowledge emerged with immune checkpoints function at immune synapses, combinatorial schedules seem to be a promising strategy. In this sense, combining chemotherapy and immunotherapy is an interesting approach in chemo-sensitive diseases that will eventually synergize and reach meaningful clinical results.

Gemcitabine is a cytotoxic drug with well-known immunostimulatory properties that include increasing antigen (neoantigens) threshold and cross-presentation (via APCs), with enhancement of T-cell response and generation of memory T cells. In addition, gemcitabine has demonstrated the ability to restore immune surveillance by reducing myeloid derived suppressor cells (MDSC) levels in murine models. Furthermore, gemcitabine-based schedules have demonstrated clinical activity in breast cancer.

Programmed death 1 (PD-1) is a molecule expressed on activated T cells that plays a major role in maintenance of T-cell tolerance limiting effector T cell responses. There are two ligands of PD-1, PD-L1 and PD-L2 (or B7-H1 and B7-H2), although PD-L1 is considered the most important one. PD-L1 is aberrantly expressed in some tumors including breast cancer, and thus it can induce immune suppression through signaling PD-1. In breast cancer PD-L1 expression (in tumor tissue and Tumor infiltrating lymphocytes (TILs)) has been shown to be correlated with worse clinicopathological data like larger tumor size, histologic grade III tumors, or negative hormone receptors. Blocking PD-1/PD-L1 interaction might have an immunogenic effect in

breast carcinoma patients. At this point, pembrolizumab is a human programmed death receptor-1 (PD-1)-blocking antibody that has gained Food and Drug Administration (FDA) approval for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab treatment.

In our opinion, there is sufficient evidence to consider that advanced breast carcinoma may be sensitive to immunotherapeutic approaches. Our proposal is based on a combination strategy with two immunostimulatory agents: gemcitabine (immunogenic apoptosis and elimination of MDSC) and pembrolizumab (blocking PD1/PD-L1 interaction) in advanced breast carcinoma (ABC) that may synergize and induce responses with long term clinical benefit.

At this point, there exist a set of immune biomarkers in peripheral blood as myeloid derived suppressor cells (MDSC), regulatory T cells (Treg), cytokines and others that can be highly informative with respect to the immune activation status of the host. This trial will include translational research that will pursue to analyze the abovementioned set of immune biomarkers in the clinical setting, looking at their basal level and monitoring their evolution at different time points during treatment with gemcitabine and pembrolizumab

This translational research may shed light on the putative mechanisms of clinical activity of this combination and identify endpoints that may predict clinical activity.

Study Drug/Medication:

Eligible patients will be enrolled and treated with:

- ✓ Pembrolizumab at a dose of 200mg as an intravenous (IV) infusion on day 1 of each 21-day cycle.

in combination with

- ✓ Gemcitabine at a dose of 1,250mg/m^2 or 1,000mg/m^2 (this dose will be explored in combination with pembrolizumab in the initial exploratory run-in-phase if necessary) as an intravenous (IV) infusion on day 1 and 8 of each 21-day cycle.

Treatment will be repeated on day 1 of each 21-day cycle until objective disease progression, clinical progression (under investigator criteria), unacceptable toxicity, death or withdrawal of consent, whichever occurs first. Patients completing 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication will stop pembrolizumab treatment (though may continue with gemcitabine). Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional pembrolizumab treatment if they progress after stopping it.

An initial exploratory run-in-phase will be performed to test the safety of the combination and determine the Recommended Phase II Dose (RP2D) of gemcitabine in combination with fixed doses of pembrolizumab.

Study Design and Treatment:

This is a multicenter phase II trial, with an initial exploratory run-in-phase, to evaluate the efficacy and safety of pembrolizumab in combination with gemcitabine in patients with HER2-negative ABC that have previously received anthracyclines and taxanes (unless clinically contraindicated). In hormone receptor positive patients, previous treatment with 2 or more lines of hormone therapy will also be required. Patients must have at least one measurable lesion that can be accurately assessed at baseline and is suitable for repeated assessment by CT, MRI or plan X-ray and a metastatic lesion accessible for biopsy. Approximately 53 patients (up to a maximum of 65 patients depending on the results of the run-in-phase) will be included in this trial.

The study will include two cohorts of patients: i) Triple Negative and ii) Luminal A+B, with an approximate 1:1 distribution between both groups.

A safety dose testing or "run-in-phase", with a 6+6 design, in which toxicity will be evaluated within the first cycle, will be performed since pembrolizumab in combination with gemcitabine has not been previously tested. Initially 6 patients will be included in the study at dose level 0 (gemcitabine at a dose of 1,250mg/m^2 as an IV infusion on day 1 and 8 of each 21-day cycle and pembrolizumab at a dose of 200mg as an IV infusion on day 1 of each 21-day cycle):

- If $\leq$ 2 patients experience Dose Limiting Toxicity (DLT), 6 additional patients will be included at the current dose level. If there is a confirmation of this dose to be safe ($\leq$ 3 patients experiencing DLT), this will be considered the RP2D and it will be used for the following recruited patients.
- If $\geq$ 3 patients experience DLT within the first 6 patients, or $\geq$ 4 within the first 12 patients included at dose level 0, a de-escalation to dose level -1 (gemcitabine at a dose of 1,000mg/m^2 as an IV infusion on day 1 and 8 of each 21-day cycle and pembrolizumab at a dose of 200mg as an IV infusion on day 1 of each 21-day cycle) will be performed. In this case, a group of 12 additional patients will be included at dose level -1, if $\geq$ 4 experience DLT, this combination will be considered too toxic and the study will be stopped. If $\leq$ 3 experience DLT with this combination, this will be considered the RP2D and it will be used for the following recruited patients.

Initially 3 patients will be allowed for inclusion simultaneously. These 3 patients will be followed closely during the first cycle to observe the occurrence of any DLT. At the times these 3 patients are completing the first cycle, the next 3 patients will be included one by one, until the first cohort is completed.

If none of these 6 patients have a DLT, up to 4 patients will be allowed for inclusion from the second cohort of 6 patients; they will follow the same procedure as in the first cohort. If one of these patients from the second cohort has a DLT, the inclusion will be in smaller groups (with a maximum number of 4 patients with DLT) and following the same procedure as in the first cohort

of 6 patients.

An internal committee will periodically review the safety data in order to take the decision to maintain or decrease the dose level. This internal committee will consist of the chief investigator, the GEICAM medical monitor and the study statistician. The meetings will be performed by teleconference to take these decisions as quickly as possible once the last patient finishes the first cycle of treatment. Other meetings will be considered ad-hoc whenever necessary (i.e when new DLTs appear).

Patients included in the run-in-phase at the same dose than that in the phase II will be considered for the phase II analysis.

Primary Objective:

- Run-in-phase: To determine the Recommended Phase II Dose (RP2D) of gemcitabine in combination with fixed doses of pembrolizumab.
- Phase II: To assess the efficacy of pembrolizumab in combination with gemcitabine in terms of Objective Response Rate (ORR) in patients with HER2-negative ABC.

Primary End-point:

- Run-in-phase: To determine the incidence rate of Dose Limiting Toxicity (DLT) within the first cycle of the combination.
- Phase II: Objective Response Rate (ORR) is defined as Complete Response (CR) plus Partial Response (PR) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

Secondary Objectives:

The following secondary objectives will be studied:

- To assess other efficacy measures of the combination in patients included in the phase II (including those in the run-in-phase at the same dose that in the phase II).
- To determine safety and tolerability of the combination in all patients included in the study.

Secondary End-points:

The following secondary end-points will be studied:

- Efficacy:
    - Progression-Free Survival (PFS) assessed according to RECIST version 1.1 by the investigator.

- Clinical Benefit Rate (CBR) defined as Complete Response (CR) plus Partial Response (PR) plus Stable Disease (SD) lasting ≥ 24 weeks according to RECIST version 1.1.
- Response Duration (RD) assessed according to RECIST version 1.1.
- Overall Survival (OS). With special interest on long term responders (i.e. alive and without disease progression after 24 months of study treatment).

- Safety will be assessed by standard clinical and laboratory tests (hematology, serum chemistry). Adverse Events (AE) grade will be defined by the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) version 4.0.

Exploratory Objectives:

The following exploratory objectives will be studied in all patients included in the study unless otherwise specified:

- To assess other efficacy measures of the combination based on immune-related (ir) response criteria in patients included in the phase II (including those in the run-in-phase at the same dose that in the phase II).
- To search for tumor tissue and peripheral blood biomarkers of clinical activity.
- To compare biomarkers data from cohorts of healthy volunteers (if available) with data from patients included in the study.

Exploratory End-points:

The following exploratory end-points will be studied:

- Efficacy:
  - ORR is defined as irCR plus irPR according to irRECIST.
  - Progression-Free Survival (PFS) assessed according to irRECIST by the investigator.
  - Clinical Benefit Rate (CBR) defined as irCR plus irPR plus irSD lasting ≥ 24 weeks according to irRECIST.
  - Response Duration (RD) assessed according to irRECIST.
- A set of immune biomarkers will be analysed and correlated with evolution of the disease and efficacy of pembrolizumab in combination with gemcitabine (ORR and other efficacy end-points: PFS, CBR and RD), paying special attention to long term responders.
- This set of immune biomarkers will be compared with those from healthy volunteers (if available).

Study population and main inclusion and exclusion criteria:

Patients with HER2-negative advanced breast cancer.

Inclusion Criteria:

Patients are eligible to be included in the study only if they **meet all** of the following criteria:

1. The patient has signed and dated the informed consent document and it has been obtained before conducting any procedure specifically for the study.
2. Female ≥ 18 years of age on day of signing informed consent.
3. Histological/cytological confirmation of breast cancer with evidence of advanced disease, not amenable to resection or radiation therapy with curative intent.
4. Documented luminal A, luminal B (HER2-negative) or triple negative disease by immunohistochemistry (IHQ) and/or *in situ* hybridization (FISH/CISH/SISH) based on local testing on the most recent tumor biopsy defined as follows:
    - **Luminal A**: tumor with **positive estrogen receptor (ER) status** (≥1% of tumor cells with ER expression) and **HER2-negative status** (IHQ score 0/1+ or negative by *in situ* hybridization defined as a HER2/CEP17 ratio < 2 or for single probe assessment a HER2 copy number < 4) and **high progesterone receptor (PgR)** (≥ 20% of tumor cells with PgR expression) and **low Ki67** (< 14%).
    - **Luminal B (HER2-negative):** tumor with **positive ER status** (≥1% of tumor cells with ER expression) and **HER2-negative status** (IHQ score 0/1+ or negative by *in situ* hybridization defined as a HER2/CEP17 ratio < 2 or for single probe assessment a HER2 copy number < 4) and either **low or negative PgR** (< 20% of tumor cells with PgR expression) and/or **high Ki67** (≥ 14%).
    - **Triple negative**: tumor with **negative hormone receptor status** (<1% of tumor cells with ER and PgR expression) and **HER2-negative status** (IHQ score 0/1+ or negative by *in situ* hybridization defined as a HER2/CEP17 ratio < 2 or for single probe assessment a HER2 copy number < 4).
5. Have at least one unidimensionally measurable lesion by RECIST 1.1.
6. Patient agrees to the collection of a metastatic tumor sample (biopsy) at the time of inclusion and at progression (whenever possible).
7. Have a performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) Performance Scale.
8. Demonstrate adequate organ function as follows (all screening labs should be performed within 7 days of study treatment initiation):

- Bone marrow:
  - Absolute Neutrophil Count (ANC) ≥ 1,500/mm^3 ($1.5x10^9$/l)
  - Platelets ≥ 100,000/mm^3 ($100x10^9$/l)
  - Hemoglobin ≥ 9g/dl or ≥ 5.6 mmol/l without transfusion or EPO dependency (within 7 days of assessment)
- Hepatic:
  - Serum total bilirubin ≤ 1.5 x Upper Limit of Normal (ULN)
  - Alkaline Phosphatase ≤ 2.5 x ULN or ≤ 5 x ULN for patients with liver metastases or bone metastases or any non-malignant bone disease
  - AST (SGOT) and ALT (SGPT) ≤ 2.5 x ULN or ≤ 5 x ULN for patients with liver metastases
  - Albumin ≥ 2.5 g/dl
- Renal:
  - Serum creatinine ≤ 1.5 x ULN or creatinine clearance ≥ 60 ml/min for patients with creatinine levels > 1.5 x ULN
- Coagulation:
  - International Normalized Ratio (INR) or Prothrombin Time (PT) ≤ 1.5 x ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.
  - Activated Partial Thromboplastin Time (aPTT) ≤ 1.5 x ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.

9. Prior treatment with anthracyclines and taxanes (unless clinically contraindicated), two or more prior lines of hormone therapy in hormone receptor positive disease, and no more than four prior chemotherapy lines for ABC.
10. At least 3 months life expectancy.
11. Patient of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study drug/medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
12. Patients of childbearing potential (see section 4.4. for definition) must be willing to use an adequate method of contraception as outlined in Section 4.4. – Contraception, for the course of the study through 120 days after the last dose of study medication.

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

the subject.

13. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

Exclusion Criteria:

Patients will be excluded from the study if they **meet any** of the following criteria:

1. HER2-positive disease by immunohistochemistry or in situ hybridation (FISH-SISH-CISH).
2. Patient is currently participating or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of study drug/medication.
3. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study day 1 or who has not recovered (i.e., ≤ grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
4. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study day 1 or who has not recovered (i.e., ≤ grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Patients with ≤ grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    - Note: If patient received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
5. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
6. 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.
7. Has hypersensitivity to pembrolizumab, gemcitabine or any of theirs excipients.
8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Patients with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and all neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
9. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other

form of immunosuppressive therapy within 7 days prior to the first dose of study drug/medication.

10. Has an 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 (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
11. Has a current or prior malignancy within the previous 5 years (other than breast cancer or adequately treated basal cell or squamous cell carcinoma of the skin or in-situ carcinoma of the cervix).
12. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.
13. Has an active infection requiring systemic therapy.
14. Has a known history of active TB (Bacillus Tuberculosis) or Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) or a known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
15. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator.
16. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
17. Patient is pregnant or breastfeeding, or expecting to conceive within the projected duration of the trial, starting with the baseline visit through 120 days after the last dose of trial treatment.

Justification of Sample size determination:

A Simon minimax two-stage design will be employed with the possibility of stopping early due to lack of response. Results from previous studies showed that gemcitabine produced a response rate of around 20%, this will be our expected H0. With the combination of pembrolizumab and gemcitabine, we expect to increase this rate to 35% what will be our H1 (an absolute increase of 15%), with an alpha error of 0.05 and a statistical power of 80%, we will need to include 53 evaluable patients in this trial. The first stage will include 31 evaluable patients, if at least 7 present a response, recruitment will continue to include the 53 evaluable patients. The null hypothesis of H0=20% will be rejected if 16 or more responses are observed in 53 patients.

Statistical Analyses:

- Demographics and Baseline Characteristics

  Standard descriptive statistics, such as the mean, median, range and proportion, will be used to summarize the patient sample and to estimate parameters of interest.

- Safety Analyses

  AEs and Serious Adverse Events (SAE) will be reported in frequency tables (overall and by intensity). The safety analysis will be performed in the population that has received at least one dose of any of the study drugs/medications.

- Efficacy Analyses

  All efficacy endpoints will be evaluated in the **Efficacy** and **ITT** populations.

- Biomarker analysis

  The biomarker analysis will be exploratory and descriptive. For continuous variables, mean, standard deviation, median, minimum and maximum values will be provided. Categorical variables will be summarized by numbers and proportions. Biomarker endpoints will be evaluated in all patients enrolled in the study with available samples.

Study Duration:

The end date of study is date of last patient´s death or the date when there is sufficient data to achieve the primary and secondary objectives and all patients have ended the study treatment, whichever comes first.

Performing exploratory objectives will be independent of the date of the end of the study.

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Abbreviations and Definitions

ABC	Advanced Breast Cancer
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT/ALAT (SGPT)	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ALC	Absolute Lymphocyte Count
aPTT	Activated Partial Thromboplastin Time
AR	Adverse Reaction
AST/ASAT (SGOT)	Aspartate Aminotransferase
BC	Breast Cancer
CB	Clinical Benefit
CBR	Clinical Benefit Rate
CI	Confidence Interval
CISH	Chromogenic In Situ Hybridization
Compliance	Adherence to all the trial-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
CNS	Central Nervous System
CR	Complete Response
CTCAE	Common Terminology Criteria for Adverse Events
CT Scan	Computed Tomography Scan
DKA	Diabetic Ketoacidosis
DLT	Dose Limiting Toxicity
eCRF	Electronic Case Report Form (sometimes referred to as Clinical Report Form). An electronic form for recording study participants' data during a clinical study, as required by the protocol.
ECG	Electrocardiogram

ECI	Event of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicines Agency
End of Study (Trial)	The end of study (trial) is the date of the last visit or last scheduled procedure shown in the Study Schedule for the last active patient in the study.
Enroll	The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned a registration number and treatment.
Enter	The act of obtaining informed consent for participation in a clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients entered into a trial are those who sign the informed consent document directly or through their legally acceptable representatives.
ER	Estrogen Receptor
ERB	Ethical review board: A board or committee (institutional, regional, or national) composed of medical professional and nonmedical members whose responsibility is to verify that the safety, welfare, and human rights of the patients participating in a clinical trial are protected.
FDA	Food and Drug Administration
FISH	Fluorescence In Situ Hybridization
GCP	Good Clinical Practice
GEICAM	Spanish Breast Cancer Research Group
HER2	Human Epidermal Growth Factor Receptor 2
HIV	Human Immunodeficiency Virus
IB	Investigator´s Brochure
ICD	Informed Consent Document
IMP	Investigational Medicinal Product
Investigator	A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
INR	International Normalized Ratio
irRC	Immuno-related Response Criteria

ITT	Intent To Treat
IV	Intravenous
Legal Representative	An individual, judicial, or other body authorized under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical trial.
MDSC	Myeloid Derived Suppressor Cells
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
OR	Objective Response
ORR	Objective Response Rate
OS	Overall Survival
Patient	A subject with a defined disease.
PD	Progressive Disease or Programmed Death depending on the context
PFS	Progression-Free Survival
PgR	Progesterone Receptor
PR	Partial Response
PT	Prothrombin Time
Q2W	Every 2 Weeks
RD	Response Duration
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended Phase II Dose
SABCS	San Antonio Breast Cancer Symposium
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SD	Stable Disease

SISH	Silver In Situ Hybridization
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1DM	Type 1 Diabetes Mellitus
TIL	T Infiltrating Lymphocyte
ULN	Upper Limit of Normal
WBC	White Blood Cell

A multicenter phase II trial to evaluate the efficacy and safety of pembrolizumab and gemcitabine in patients with HER2-negative Advanced Breast Cancer (ABC)

"**PANGEA-Breast**"

1. Introduction

1.1. Overview of Breast Cancer

Breast cancer (BC) represents one of the most frequent cancers in general population and the first malignant tumor in women worldwide. In Europe, there were an estimated of 464,000 new cases of breast cancer in 2012, with a mortality of 131.000 cases (1). Specifically in Spain, incidence is around 26.000 new cases per year and although a steady decrease in mortality has been demonstrated in the last decades, nearly 20% of the patients relapse and ultimately die of breast cancer, which represent a mortality of approximately 6000 women per year in Spain (2).

1.2. Treatment options for Advanced Breast Cancer

Treatment options for advanced breast cancer (ABC) are numerous and varied, however new and better therapies are clearly needed, especially in triple negative tumors and in hormone-sensitive but heavily pretreated disease, a challenging situation where impact of the current treatments in overall survival is relatively low (3).

1.3. Immune system and tumors

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 (4-11). Immune edition has been recently recognized as a highly relevant hallmark of cancer, which may impact and contribute notably to the different evolution of almost all types of tumors (12,13).

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, so playing a major role in maintenance of T-cell tolerance limiting effector T cell responses (14,15). PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and PD-L2, or also named B7-H1 and B7-H2). The structure of murine PD-1 has been resolved (16). 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 (17). The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 (18) as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells (19). Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells (20), as well as subsets of macrophages (21) and dendritic cells (22). The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types (15), 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. Based on that, PD-L1 is considered more important than PD-L2 in solid tumors. Although healthy organs express little (if any) PD-L1, a variety of cancers, including breast cancer were demonstrated to aberrantly express abundant levels of this T-cell inhibitor (23, 24), and thus it can induce immune suppression through signaling PD-1 (15). 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.

1.3.1. Immune system and Breast cancer

In breast cancer many evidences have emerged in the last few years highlighting the role of PD-L1 expression in tumor tissue and tumor infiltrating lymphocytes (TILs). In fact PD-L1 expression and TILs have been shown to be correlated with worse clinicopathological data, like larger tumor size, histologic grade III tumors, or negative hormone receptors (25). It has been reported that high or low density of TILs infiltration may have an impact in disease free survival and overall survival in early breast cancer, especially for the triple negative and HER2-positive subtypes (26). A higher TILs infiltration seems to be related to a successful immune response of the host, that usually translate into better long term outcomes in the adjuvant setting (prognostic factor), and a higher probability to obtain a pathologic complete response in the neoadjuvant scenario (predictive factor) (27-29). Therefore, available data support the hypothesis of an immune mediated antitumor activity in breast carcinoma. Blocking PD-1/PD-L1 interaction might have an immunogenic effect in breast carcinoma patients and several lines of research are ongoing in this way.

1.4. Overview of Pembrolizumab

New immunotherapeutic drugs (monoclonal antibodies like anti-CTLA4, anti-PD1, anti-PD-L1, etc) are entering into the clinic with unexpected successful results in a broad spectrum of tumors that are apparently unrelated (melanoma, non-small cell lung cancer, bladder cancer and others) (14).

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.

A summary of the most important clinical data is included in the next sections; please refer to the Investigator´s Brochure (IB) for preclinical information and additional clinical data (30).

1.4.1. Efficacy of pembrolizumab in solid tumors

Pembrolizumab dose was assessed in the phase I KEYNOTE-001 that consisted of a first-in-human dose-finding cohort (part A) that assessed pembrolizumab given intravenously at 1 mg/kg, 2 mg/kg, 3 mg/kg, and 10 mg/kg once every 2 weeks (Q2W) or every 3 weeks (Q3W) (31) and several expansion cohorts exploring the efficacy and safety v of several pembrolizumab doses and schedules in patients with advanced melanoma (parts B and D) and non-small cell lung cancer (NSCLC) (parts C and F).

In the melanoma expansion cohorts 655 patients were included that were either ipilimumab-pretreated or naïve were treated with 3 different pembrolizumab dose regimens (2 mg/kg Q3W, 10 mg/kg Q3W, and 10 mg/kg Q2W) in a non-randomized (32) and a randomized fashion (33-35).

The overall response rate of pembrolizumab in patients from the non-randomized cohorts (n=135) were 38% as per RECIST criteria and 37% by immune-related response criteria (irRC) without any difference found between the ipilimumab-pretreated or naïve patients. Numerical differences in ORR were seen between doses and schedules, although the 95% confidence intervals (CIs) largely overlapped; ORR was 52% (95 % CI: 38–66) at 10 mg/kg Q2W, 27% (95 % CI: 15–42) at 10 mg/kg Q3W, and 25% (95 % CI: 9–49) at 2 mg/kg Q3W.

One of the randomized cohorts of KEYNOTE-001 included 173 ipilimumab-refractory patients who were randomly assigned to pembrolizumab 2 mg/kg Q3W or 10 mg/kg Q3W (33). ORR at both doses was 26% as per RECIST criteria. In the ipilimumab-naive randomized cohort, 103 patients were randomly assigned to receive pembrolizumab 2 mg/kg or 10 mg/kg Q3W (34). ORR per RECIST v1.1 was 33% in the 2-mg/kg group and 40% in the 10-mg/kg group (P = 0.48). The final randomized melanoma cohort of KEYNOTE-001 included 244 patients with ipilimumab-naive or ipilimumab-treated disease who were randomly assigned to pembrolizumab 10 mg/kg Q2W or Q3W to further explore response and outcome at these

schedules (35). ORR was 31% in the Q3W arm and 35% in the Q2W arm, indicating no difference in ORR between schedules (P = 0.5052).

All this data together compared favorably to historical response rates for available treatments for melanoma. For example, the largest randomized clinical trial in previously treated advanced melanoma patients, in which carboplatin and paclitaxel were used in the control arm, and sorafenib plus carboplatin and paclitaxel in the experimental arm, produced a response rate of 11% and 12%, respectively (36). This data shows significant activity regardless of prior ipilimumab treatment (although those ipilimumab naïve appear to have higher response rates). Of importance is the fact that the studies did not show a statistically significant difference in activity between doses and schedules.

In the phase II randomized KEYNOTE-002 study, 540 patients previously treated with ipilimumab or BRAF/MEK inhibitors (if BRAF positive) were randomize to receive pembrolizumab (two arms with two schedules of 2 or 10 mg/kg Q3W, respectively) or chemotherapy (best dealers choice) (37). PFS was improved in patients assigned to pembrolizumab 2 mg/kg (HR 0.57, 95%CI 0.45-0.73; p<0.0001) and pembrolizumab 10 mg/kg (0.50, 95%CI 0.39-0.64; p<0.0001) compared with those assigned to chemotherapy. 6-month progression-free survival was 34% (95%CI 27-41) in the pembrolizumab 2 mg/kg group, 38% (95%CI 31-45) in the 10 mg/kg group, and 16% (95%CI 10-22) in the chemotherapy group. Treatment with pembrolizumab lead to an ORR that was >4 fold higher (21.1%; 95%CI 15.4, 27.8 and 25.4%; 95%CI:19.2,32.4, for the 2 and 10 mg/Kg respectively) than the response rate of the chemotherapy control arm (4.5%; 95%CI 1.9,8.6). This difference was highly statistically significant, with a one sided p-value of <0.0001.

Based on this data, KeytrudaTM (pembrolizumab) at a dose of 2 mg/kg Q3W has been approved by the FDA and EMA for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

Very recently, the results from the KEYNOTE-006 trial in melanoma patients that have not been previously pretreated with ipilimumab, have also been reported (38). This is a randomized, controlled, phase III study, in which 834 patients with advanced melanoma were randomized in a 1:1:1 ratio to receive pembrolizumab (at a dose of 10 mg/Kg) every 2 weeks or every 3 weeks or four doses of ipilimumab (at 3 mg/Kg) every 3 weeks. The estimated 6-month progression-free-survival rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab (HR for disease progression, 0.58; P<0.001 for both pembrolizumab regimens versus ipilimumab; 95% confidence intervals [CIs], 0.46 to 0.72 and 0.47 to 0.72, respectively). The estimated 12-month survival rates were 74.1%, 68.4%, and 58.2%, respectively (HR for death for pembrolizumab every 2 weeks, 0.63; 95% CI, 0.47 to 0.83; P=0.0005; HR for pembrolizumab every 3 weeks, 0.69; 95% CI, 0.52 to 0.90; P=0.0036). The ORR was improved with pembrolizumab administered every 2 weeks (33.7%)

and every 3 weeks (32.9%), as compared with ipilimumab (11.9%) (P<0.001 for both comparisons). Responses were ongoing in 89.4%, 96.7%, and 87.9% of patients, respectively, after a median follow-up of 7.9 months. Efficacy was similar in the two pembrolizumab groups.

Pembrolizumab has also shown activity in non-small cell lung cancer in 495 patients (394 previosuly treated and 101 treatment naïve) included in the KEYNOTE-001 study receiving two different doses of either 2 or 10 mg/Kg every 3 weeks or 10 mg/Kg every 2 weeks (39). The response rate was 19.4%, in all patients, with and ORR of 24.8% (95% CI: 16.7–34.3) in treatment-naive patients and 18.0% (95% CI: 14.4–22.2) in previously treated patients. Pembrolizumab demonstrated an ORR of 33.3% (95% CI: 4.3–77.7) at 2 mg/kg Q3W, 19.2% (95% CI: 14.8–24.2) at 10 mg/kg Q3W, and 19.3% (95% CI: 14.1–25.4) at 10 mg/kg Q2W. Other efficacy variables showed a median duration of response of 12.5 months (10.4 months in previously treated patients and 23.3 months in treatment naïve patients), a median PFS of 3.7 months (3 months in previously treated patients and 6 months in treatment naïve patients) and a median overall survival of 12.0 months (9.3 months in previously treated patients and 16.2 months in treatment naïve patients. PD-L1 expression was assessed in a set of these patients (training set of 182 patients) and a cut-off of at least 50% of tumor cells was selected. Among patients with a proportion score of at least 50% in the validation group (n=313), the response rate was 45.2%, the median progression-free survival was 6.3 months and the median overall survival was not reached (better than those with <50% of tumor cells expressing PD-L1).

First data with pembrolizumab in breast cancer were communicated in December 2014 at the San Antonio Breast Cancer Symposium (SABCS 2014) in a population of heavily pretreated (≥ 2 treatments for metastatic disease in 65.7% of the cases) ABC women with triple negative and PD-L1+ tumors. Pembrolizumab was administered at 10 mg/kg every 2 weeks to a total of 32 included patients. An overall response rate of 18.5% (5/27 evaluable patients) and clinical benefit rate of 44.4% (including 7 patients with SD) and PFS of 1.9 months were reported. Anti-PD1 treatment was safe; adverse events of any grade were observed in 56% of patients; grade 3 events were observed in 12.5% and grade 4 in 3.1%. Three patients (9.4%) had a serious adverse event, one of which resulted in death due to disseminated intravascular coagulation (40). These data have served as a proof of principle of activity for anti-PD1 therapies in breast cancer.

1.4.2. Safety of pembrolizumab

Toxicity reported to date, primarily in melanoma patients, has been manageable and not treatment limiting in the majority of patients. The most common AEs have been fatigue, rash, pruritus, arthralgia, amylase elevation, and diarrhea. AEs are generally immune-related but manageable following the guidelines drawn from previous experience with ipilimumab, mainly with corticosteroids and dose interruptions.

Common AEs of any grade and Grade 3 or above AEs detected in patients enrolled in KEYNOTE-001 are described in Tables 1 and 2 respectively. The incidence of grade ≥3 AEs was 14% in the nonrandomized melanoma cohorts and 12% in the randomly assigned ipilimumab-refractory patients, the most prevalent were pneumonitis, diarrhea, hepatitis, and endocrine-related AEs such as hyper- or hypothyroidism.

Table 1. Adverse events with incidence ≥5 % observed in patients from KEYNOTE-001 and KEYNOTE-006 trials.

AE, %	Nonrandomized and randomized cohorts KEYNOTE-001 (n = 411) [16]	NSCLC cohorts KEYNOTE-001 (n = 495) [22]	KEYNOTE-006 (melanoma, 10 mg/kg Q2W, n = 278) [21]	KEYNOTE-006 (melanoma, 10 mg/kg Q3W, n = 277) [21]
Fatigue	36	19	21	19
Pruritus	24	11	14	14
Rash	20	10	15	13
Arthralgia	16	9	9	12
Diarrhea	16	8	17	14
Nausea	12	8	10	11
Vitiligo	11	NR	9	11
Asthenia	9	5	12	11
Cough	9	2	4	4
Myalgia	9	3	7	2
Headache	8	2	3	2
Hypothyroidism	8	7	10	9
Decreased appetite	7	11	6	7
Dyspnea	7	4	1	3
Chills	6	2	1	0
Pyrexia	6	4	4	1
ALT increase	5	2	4	1
Pneumonitis	3	4	<1	2
Hyperthyroidism	1	2	7	3
Colitis	<1	NR	2	4
Hepatitis	<1	NR	1	2
Hypophysitis	NR	NR	<1	<1
Nephritis	NR	NR	0	<1

KEYNOTE-001 included melanoma and lung cohorts; KEYNOTE-006 included patients with melanoma
Numbers given as percentages where available
Abbreviations: *AE* adverse event; *ALT* alanine aminotransferase; *DRAEs* drug-related AEs; *NR* not reported; *NSCLC* non-small cell lung cancer

Table 2. Incidence of grade ≥ 3 AEs in patients from KEYNOTE-001 and KEYNOTE-006

AE, %	Nonrandomized and randomized cohorts (n = 411) [16]	NSCLC cohorts (n = 495) [22]	KEYNOTE-006 (melanoma, 10 mg/kg Q2W, n = 278) [21]	KEYNOTE- 006 (melanoma,10 mg/kg Q3W, n = 277) [21]
Fatigue	2	<1	0	<1
ALT increase	<1	<1	0	<1
Colitis	<1	NR	1	3
Decreased appetite	<1	1	0	0
Diarrhea	<1	<1	3	1
Dyspnea	<1	4	0	<1
Headache	<1	NR	0	0
Hepatitis	<1	NR	1	2
Hyperthyroidism	<1	NR	0	0
Hypophysitis	<1	NR	<1	<1
Hypothyroidism	<1	<1	<1	0
Nausea	<1	<1	0	<1
Pneumonitis	<1	2	0	<1
Pruritus	<1	0	0	0
Rash	<1	<1	0	0
Arthralgia	0	<1	0	<1
Asthenia	0	1	<1	0

KEYNOTE-001 included melanoma and lung cohorts; KEYNOTE-006 included patients with melanoma
Numbers given as percentages where available
Abbreviations: *AE* adverse event; *ALT* alanine aminotransferase; *DRAE* drug-related AEs; *NR* not reported; *NSCLC* non-small cell lung cancer

In KEYNOTE-006 the pembrolizumab arms had less toxicity compared with ipilimumab; incidence of grade ≥3 toxicity was 13.3%, 10.1%, and 19.9% for the 10 mg/kg Q2W vs 10 mg/kg Q3W doses of pembrolizumab and ipilimumab, respectively. The rate of drug discontinuation secondary to AEs for these groups was 4.0%, 6.9%, and 9.4%, respectively. Common AEs observed with pembrolizumab were fatigue, diarrhea, hyperthyroidism, hypothyroidism, rash, and pruritus; grade ≥3 diarrhea occurred in >1 % of patients (2.5 % and 1.1%, respectively, for Q2W vs Q3W schedules). Grade ≥3 colitis occurred in 1.4% and 2.5% and grade ≥3 hepatitis in 1.1% and 1.8% at the Q2W and Q3W schedules, respectively, detailed in Tables 2 and 3.

Lung patients treated on KEYNOTE-001 had a similar safety profile to that observed in patients with melanoma. The incidence of AEs of grade ≥3 was 9.5%, with pneumonitis (1.8%), dyspnea (3.8%), decreased appetite (1%), and asthenia (1%) having the highest frequencies. Pneumonitis of any grade occurred in 3.6% of patients. To date, no specific associations in patients with NSCLC have been reported between the risk of pneumonitis and previous or subsequent radiotherapy after progression on an anti-PD-1/PD-L1 agent. There was 1 treatment-related death (pneumonitis). Median follow-up duration in all reported and published trial cohorts has been short, and mature data on long-term toxicity are awaited. The kinetics of toxicity will be important in managing patients on pembrolizumab, especially in patients undergoing continued treatment within the context of durable CR or PR. Important

issues for future investigation include how the profile of AEs will alter with combinatorial immune or multimodality therapies, such as vaccines and radiation therapy.

1.4.3. Rationale for Dose Selection/Regimen/Modification

The dose escalation portion of KEYNOTE-001 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, as a consequence no MTD was identified. This first in human study of pembrolizumab 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) (31). Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of pembrolizumab 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 pembrolizumab 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. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200mg 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 pembrolizumab 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 200mg 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 200mg 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.

1.5. Immunotherapy in combination with chemotherapy

At this point and due to the new knowledge generated with immune checkpoints function at immune synapses, combination schedules seem to be a promissory strategy, so that "non-inflamed" tumors in a basal state may switch to an "inflamed" condition, triggering the danger signal to activate the immune system of the host (41,42). Especially in chemo-sensitive diseases, the combination of chemotherapy and immunotherapy seems to be an interesting approach that will eventually synergize and reach meaningful clinical results (43).

1.5.1. Gemcitabine

Gemcitabine is a cytotoxic drug with well-known immunostimulatory properties that include increasing antigen (neoantigens) threshold and cross-presentation (via APCs), with enhancement of T-cell response and generation of memory T cells (44). In addition, gemcitabine has demonstrated the ability to restore immune surveillance by reducing myeloid derived suppressor cells (MDSC) levels in murine models (45).

Furthermore, gemcitabine-based schedules have demonstrated clinical activity in breast cancer (46-48) achieving response rates of around 16 to 29%.

1.6. Study Rationale

At the present time there is enough preclinical and clinical evidence to consider that ABC may be sensitive to immunotherapeutic approaches. This trial is based on a combination strategy with two immunostimulatory agents: gemcitabine (immunogenic apoptosis and elimination of MDSC) and pembrolizumab (blocking PD1/PD-L1 interaction) in ABC in the search of a synergism that may induce responses with long term clinical benefit. Gemcitabine and pembrolizumab as single therapies are safe with very a very low rate of serious adverse events (SAE), therefore combination of both drugs might be warranted on efficacy and toxicity grounds. Nevertheless, we propose to perform a safety dose testing phase or run-in-phase since pembrolizumab in combination with gemcitabine has not been previously tested.

This trial includes a translational sub-study that will pursue to analyze a set of immune biomarkers in peripheral blood, looking at their basal level and monitoring their evolution at different time points during treatment with gemcitabine and pembrolizumab. In ABC, immune response assessment in tissue is challenging as biopsies are at many times not easily accessible or/and risky for the patients. At this point, there exist a set of immune biomarkers in peripheral blood as myeloid derived suppressor cells (MDSC), regulatory T cells (Treg), cytokines and others that can be highly informative with respect to the immune activation status of the host (49,50). This translational research may shed light on the putative mechanisms of the eventual efficacy of this combination and ultimately identify immune biomarkers that may predict clinical activity.

2. Objectives

2.1. Primary Objective

- Run-in-phase: To determine the Recommended Phase II Dose (RP2D) of gemcitabine in combination with fixed doses of pembrolizumab.
- Phase II: To assess the efficacy of pembrolizumab in combination with gemcitabine in terms of Objective Response Rate (ORR) in patients with HER2-negative ABC.

2.2. Primary End-point

- Run-in-phase: To determine the incidence rate of Dose Limiting Toxicity (DLT) within the first cycle of the combination.
- Phase II: Objective Response Rate (ORR) is defined as Complete Response (CR) plus Partial Response (PR) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

2.3. Secondary Objectives

The following secondary objectives will be studied:

- To assess other efficacy measures of the combination in patients included in the phase II (including those in the run-in-phase at the same dose that in the phase II).
- To determine safety and tolerability of the combination in all patients included in the study.

2.4. Secondary End-points

The following secondary end-points will be studied:

- Efficacy:
    - Progression-Free Survival (PFS) assessed according to RECIST version 1.1 by the investigator.
    - Clinical Benefit Rate (CBR) defined as Complete Response (CR) plus Partial Response (PR) plus Stable Disease (SD) lasting ≥ 24 weeks according to RECIST version 1.1.
    - Response Duration (RD) assessed according to RECIST version 1.1.
    - Overall Survival (OS). With special interest on long term responders (i.e. alive and without disease progression after 24 months of study treatment).

- Safety will be assessed by standard clinical and laboratory tests (hematology, serum chemistry). Adverse Events (AE) grade will be defined by the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) version 4.0.

2.5. Exploratory Objectives

The following exploratory objectives will be studied in all patients included in the study unless otherwise specified:

- To assess other efficacy measures of the combination based on immune-related (ir) response criteria in patients included in the phase II (including those in the run-in-phase at the same dose that in the phase II).
- To search for tumor tissue and peripheral blood biomarkers of clinical activity.
- To compare biomarkers data from cohorts of healthy volunteers (if available) with data from patients included in the study.

2.6. Exploratory End-points

The following exploratory end-points will be studied:

- Efficacy:
    - ORR is defined as irCR plus irPR according to irRECIST.
    - Progression-Free Survival (PFS) assessed according to irRECIST by the investigator.
    - Clinical Benefit Rate (CBR) defined as irCR plus irPR plus irSD lasting ≥ 24 weeks according to irRECIST.
    - Response Duration (RD) assessed according to irRECIST.
- A set of immune biomarkers will be analysed and correlated with evolution of the disease and efficacy of pembrolizumab in combination with gemcitabine (ORR and other efficacy end-points: PFS, CBR and RD), paying special attention to long term responders.
- This set of immune biomarkers will be compared with those from healthy volunteers (if available).

3. Investigational Plan

3.1. Study Design

This is a multicenter phase II trial to evaluate the efficacy and safety of pembrolizumab in combination with gemcitabine in patients with HER2-negative ABC that have previously received anthracyclines and taxanes (unless clinically contraindicated). In hormone receptor positive patients, previous treatment with 2 or more lines of hormone therapy will also be required. Patients must have at least one measurable lesion that can be accurately assessed at baseline and is suitable for repeated assessment by CT, MRI or plan X-ray and a metastatic lesion accessible for biopsy.

The study will include two cohorts of patients: i) Triple Negative and ii) Luminal A+B, with an approximate 1:1 distribution between both groups.

An initial exploratory run-in-phase will be performed to test the safety of the combination and determine the RP2D of gemcitabine in combination with fixed doses of pembrolizumab.

Approximately 53 patients (up to a maximum of 65 patients depending on the results of the run-in-phase) will be included in this trial.

3.1.1. Run-in-phase

A safety dose testing or "run-in-phase", with a 6+6 design, in which toxicity will be evaluated within the first cycle, will be performed. Initially 6 patients will be included in the study at dose level 0 (gemcitabine at a dose of 1,250mg/m^2 as an IV infusion on day 1 and 8 of each 21-day cycle and pembrolizumab at a dose of 200mg as an IV infusion on day 1 of each 21-day cycle):

- If ≤ 2 patients experience Dose Limiting Toxicity (DLT), 6 additional patients will be included at the current dose level. If there is a confirmation of this dose to be safe (≤ 3 patients experiencing DLT), this will be considered as RP2D and it will be used for the following recruited patients.
- If ≥ 3 patients experience DLT within the first 6 patients, or ≥ 4 within the first 12 patients included at dose level 0, a de-escalation to dose level -1 (gemcitabine at a dose of 1,000mg/m^2 as an IV infusion on day 1 and 8 of each 21-day cycle and pembrolizumab at a dose of 200mg as an IV infusion on day 1 of each 21-day cycle) will be performed. In this case, a group of 12 additional patients will be included at dose level -1, if ≥ 4 experience DLT this combination will be considered too toxic and the study will be stopped. If ≤ 3 experience DLT with this combination, this will be considered the RP2D and it will be used for the following recruited patients.

Initially 3 patients will be allowed for inclusion simultaneously. These 3 patients will be followed closely during the first cycle to observe the occurrence of any DLT. At the times

these 3 patients are completing the first cycle, the next 3 patients will be included one by one, until the first cohort is completed.

If none of these 6 patients have a DLT, up to 4 patients will be allowed for inclusion from the second cohort of 6 patients; they will follow the same procedure as in the first cohort. If one of these patients from the second cohort has a DLT, the inclusion will be in smaller groups (with a maximum number of 4 patients with DLT) and following the same procedure as in the first cohort of 6 patients.

An internal committee will periodically review the safety data in order to take the decision to maintain or decrease the dose level. This internal committee will consist of the chief investigator, the GEICAM medical monitor and the study statistician. The meetings will be performed by teleconference to take these decisions as quickly as possible once the last patient finishes the first cycle of treatment. Other meetings will be considered ad-hoc whenever necessary (i.e when new DLTs appear).

Definition of the Dose Limiting Toxicity (DLT):

DLT is defined as the occurrence of any of the following adverse events or abnormal laboratory value (graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0), assessed as possibly, probably or definitively related to study drug/medication, occurring within the first cycle of study treatment:

Hematologic toxicities:

- Any Grade 4 thrombocytopenia or neutropenia lasting > 7 days.

Non-hematologic toxicities:

- Episcleritis, uveitis, or iritis of Grade 2 or higher.
- Any Grade 4 toxicity.
- Any Grade 3 toxicity EXCLUDING:
  - Nausea, vomiting, or diarrhea controlled by medical intervention within 72 hours.
  - Grade 3 rash in the absence of desquamation, no mucosal involvement, does not require steroids, and resolves to Grade 1 by the next scheduled dose of pembrolizumab.
  - Transient Grade 3 Aspartate Transaminase (AST/SGOT) or Alanine Transaminase (ALT/SGPT) elevation, defined as no more than 3 days with or without steroid use.
- Discontinuation or delay of more than 2 weeks of any study drug/medication due to treatment-related AE.

Patients will continue to receive the study treatment until objective disease progression, clinical progression (under investigator criteria), unacceptable toxicity, death or withdrawal of consent, whichever occurs first, this is the active treatment phase. Patients discontinuing the study treatment will enter the follow-up phase. Patients completing 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication will stop pembrolizumab treatment (though may continue with gemcitabine).

Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional pembrolizumab treatment if they progress after stopping it provided they meet the requirements detailed in Section 3.1.4

3.1.2. Phase II cohort

Eligible patients will be enrolled and treated with pembrolizumab *in combination with g*emcitabine at the RP2D. Patients included in the run-in-phase at the same dose that the phase II will be considered for the phase II analysis.

Patients will continue to receive the study treatment until objective disease progression, clinical progression (under investigator criteria), unacceptable toxicity, death or withdrawal of consent, whichever occurs first, this is the active treatment phase. Patients discontinuing the study treatment will enter the follow-up phase. Patients completing 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication will stop pembrolizumab treatment (though may continue with gemcitabine).

Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional pembrolizumab treatment if they progress after stopping it provided they meet the requirements detailed in Section 3.1.4

3.1.3. Disease Assessments

Disease assessments will be performed at baseline and every 9 weeks (± 1 week) from the start of study treatment. All lesion measurements must be recorded in the eCRF. Disease assessment for all patients at baseline will include:

- ✓ CT scan or MRI of the chest, abdomen and pelvis (CAP).
- ✓ Bone scan is mandatory if the patient has bone disease or if there is any suspicious of bone metastases. Any suspicious abnormalities (i.e., hotspots) identified on the bone scans at baseline must be confirmed by X-ray, CT scan with bone windows or MRI.
- ✓ Brain CT scan or MRI is mandatory if the patient has any suspicious of central nervous system (CNS) metastases.
- ✓ CT scan or MRI of any other site of disease as clinically indicated.

- ✓ Clinical assessment of superficial disease which will include photographs of all superficial metastatic lesions.

The method chosen to confirm the bone metastases at baseline (X-ray, CT scan with bone windows or MRI) must follow the same assessment schedule as for measurable lesions, every 9 weeks (± 1 week) from the start of study treatment. Patients with bone lesions identified at baseline will repeat the bone scans as clinically indicated (i.e., patient describes new or worsening bone pain, or has increasing alkaline phosphatase level, or other signs and symptoms of new/progressing bone metastases) and to confirm a complete response.

Each assessment will be performed as scheduled according to the calendar regardless of any dosing delay to prevent the introduction of bias into the assessment of efficacy. Failure to perform any of the required disease assessments will result in the inability to determine disease status for that time point. Tumor assessments will be performed until radiographically and/or clinically (i.e., for photographed or palpable lesions) documented Progressive Disease (PD) as per RECIST v.1.1 or irRECIST v.1.0 (if PD is not clear, an internal committee will review the case) or initiation of new anticancer therapy or discontinuation of patient from overall study participation (e.g., death, patient's request, lost to follow up), whichever occurs first.

Efficacy analyses will be performed using the local radiologist's/investigator's tumor assessments as primary data source.

Patients discontinuing the tumor assessments for the primary endpoint will enter a follow up period during which survival and new anticancer therapy information will be collected every 6 months from the last tumor assessment. At the time of the OS survival analysis all patients should have updated information in the eCRF with a maximum time window of 12 weeks to the cut-off date. The follow up period will conclude at the time of the final OS analysis.

3.1.4. Second Course Phase (Pembrolizumab retreatment Period)

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- Either
  - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
  - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy
  - Received at least two cycles with pembrolizumab beyond the date when the initial CR was declared
- OR

- Had SD, PR or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab (except for the continuation of gemcitabine)
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Inclusion criteria number 8
- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subject of childbearing potential (see section 4.4. for definition) must be willing to use an adequate method of contraception as outlined in Section 4.4. – Contraception, for the course of the study through 120 days after the last dose of study medication.

  Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation in this second course phase or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

3.2. Duration of the study

It is estimated that the accrual will be completed approximately in 18 months.

All patients included will receive study therapy until objective disease progression, clinical progression (under investigator criteria), unacceptable toxicity, death or withdrawal of consent, whichever occurs first, this is the active treatment phase. Patients discontinuing the study treatment will enter the follow-up phase. Patients completing 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication will stop pembrolizumab treatment (though may continue with gemcitabine). Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional pembrolizumab treatment if they progress after stopping it.

For safety reasons all patients will have a visit 30 (+/-5) days after finishing treatment with the study drug/medication.

The end date of study is date of last patient´s death or the date when there is sufficient data to achieve the primary and secondary objectives and all patients have ended the study treatment, whichever comes first.

Performing exploratory objectives will be independent of the date of the end of the study.

4. Study Population

4.1. Inclusion Criteria

Patients are eligible to be included in the study only if they **meet all** of the following criteria:

1. The patient has signed and dated the informed consent document and it has been obtained before conducting any procedure specifically for the study.
2. Female ≥ 18 years of age on day of signing informed consent.
3. Histological/cytological confirmation of breast cancer with evidence of advanced disease, not amenable to resection or radiation therapy with curative intent.
4. Documented luminal A, luminal B (HER2-negative) or triple negative disease by immunohistochemistry (IHQ) and/or *in situ* hybridization (FISH/CISH/SISH) based on local testing on the most recent tumor biopsy defined as follows:
    - **Luminal A**: tumor with **positive estrogen receptor (ER) status** (≥1% of tumor cells with ER expression) and **HER2-negative status** (IHQ score 0/1+ or negative by *in situ* hybridization defined as a HER2/CEP17 ratio < 2 or for single probe assessment a HER2 copy number < 4) and **high progesterone receptor (PgR)** (≥ 20% of tumor cells with PgR expression) and **low Ki67** (< 14%).
    - **Luminal B (HER2-negative):** tumor with **positive ER status** (≥1% of tumor cells with ER expression) and **HER2-negative status** (IHQ score 0/1+ or negative by *in situ* hybridization defined as a HER2/CEP17 ratio < 2 or for single probe assessment a HER2 copy number < 4) and either **low or negative PgR** (< 20% of tumor cells with PgR expression) and/or **high Ki67** (≥ 14%).
    - **Triple negative**: tumor with **negative hormone receptor status** (<1% of tumor cells with ER and PgR expression) and **HER2-negative status** (IHQ score 0/1+ or negative by *in situ* hybridization defined as a HER2/CEP17 ratio < 2 or for single probe assessment a HER2 copy number < 4).
5. Have at least one unidimensionally measurable lesion by RECIST 1.1.
6. Patient agrees to the collection of a metastatic tumor sample (biopsy) at the time of inclusion and at progression (whenever possible).
7. Have a performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) Performance Scale.
8. Demonstrate adequate organ function as follows (all screening labs should be performed within 7 days of study treatment initiation):
    - Bone marrow:

- Absolute Neutrophil Count (ANC) ≥ 1,500/mm^3 ($1.5x10^9$/l)
- Platelets ≥ 100,000/mm^3 ($100x10^9$/l)
- Hemoglobin ≥ 9g/dl or ≥ 5.6 mmol/l without transfusion or EPO dependency (within 7 days of assessment)

o Hepatic:

- Serum total bilirubin ≤ 1.5 x Upper Limit of Normal (ULN)
- Alkaline Phosphatase ≤ 2.5 x ULN or ≤ 5 x ULN for patients with liver metastases or bone metastases or any non-malignant bone disease
- AST (SGOT) and ALT (SGPT) ≤ 2.5 x ULN or ≤ 5 x ULN for patients with liver metastases
- Albumin ≥ 2.5 g/dl

o Renal:

- Serum creatinine ≤ 1.5 x ULN or creatinine clearance ≥ 60 ml/min for patients with creatinine levels > 1.5 x ULN

o Coagulation:

- International Normalized Ratio (INR) or Prothrombin Time (PT) ≤ 1.5 x ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.
- Activated Partial Thromboplastin Time (aPTT) ≤ 1.5 x ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.

9. Prior treatment with anthracyclines and taxanes (unless clinically contraindicated), two or more prior lines of hormone therapy in hormone receptor positive disease, and no more than four prior chemotherapy lines for ABC.
10. At least 3 months life expectancy.
11. Patient of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study drug/medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
12. Patients of childbearing potential (see section 4.4. for definition) must be willing to use an adequate method of contraception as outlined in Section 4.4. – Contraception, for the course of the study through 120 days after the last dose of study medication.

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

13. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

4.2. Exclusion Criteria

Patients will be excluded from the study if they **meet any** of the following criteria:

1. HER2-positive disease by immunohistochemistry or in situ hybridation (FISH-SISH-CISH).
2. Patient is currently participating or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of study drug/medication.
3. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study day 1 or who has not recovered (i.e., ≤ grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
4. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study day 1 or who has not recovered (i.e., ≤ grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Patients with ≤ grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    - Note: If patient received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
5. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
6. 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.
7. Has hypersensitivity to pembrolizumab, gemcitabine or any of theirs excipients.
8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Patients with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and all neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

9. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug/medication.
10. Has an 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 (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
11. Has a current or prior malignancy within the previous 5 years (other than breast cancer or adequately treated basal cell or squamous cell carcinoma of the skin or in-situ carcinoma of the cervix).
12. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.
13. Has an active infection requiring systemic therapy.
14. Has a known history of active TB (Bacillus Tuberculosis) or Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) or a known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
15. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator.
16. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
17. Patient is pregnant or breastfeeding, or expecting to conceive within the projected duration of the trial, starting with the baseline visit through 120 days after the last dose of trial treatment.

4.3. Discontinuations

4.3.1. Discontinuation of Study Drug/Medication

The criteria for enrollment must be followed explicitly. If a patient who does not meet enrollment criteria is inadvertently enrolled, that patient should be discontinued from the study drug/medication, but can be allowed to continue in the study in order to provide the follow-up data needed for the analysis of the entire population. An exception may be granted if the patient, in the opinion of the investigator, is having benefit from the study drug/medication. In these rare cases, the investigator must obtain documented approval from GEICAM to allow the patient to continue to receive the study drug/medication.

Patients can be discontinued from the study therapy in the following circumstances:

- Patient's own request.
- Unacceptable toxicity as defined in the protocol.
- Tumor progression/recurrence as defined in the protocol.
- Any clinical AE, laboratory abnormality or inter-current illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the patient.
- Pregnancy:
    - Instruct to contact the investigator or study staff immediately if they suspect they might be pregnant.
    - The investigator must immediately notify GEICAM if a study patient becomes pregnant.
- Termination of the study by GEICAM.
- Physician's decision, including need of other anti-cancer therapy, not specified in the protocol.
- If the patient is non-compliant with study procedures.
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g. infectious disease) illness.
- All patients will be discontinued from the active treatment phase and entered into the follow up phase in case of a delay of more than 12 weeks or permanent discontinuation of both study treatments.
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later. In this case, the investigator may decide to continue the treatment with gemcitabine if considers this is in the benefit for the patient, in this case the patient will be maintained in the active treatment phase.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional pembrolizumab treatment if they progress after stopping it provided they meet the requirements detailed in Section 3.1.4

If possible, and after the permanent discontinuation of treatment, the patients will be assessed using the procedure normally planned for the last dosing day with the study treatment.

All permanent treatment discontinuation should be recorded by the investigator in the eCRF when considered as confirmed.

4.3.2. Discontinuation of Pembrolizumab after CR

Discontinuation of pembrolizumab may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with this therapy and had at least two cycles with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab (except for the continuation of gemcitabine), the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 3.1.4

4.3.2. Discontinuation of Study Sites

Study site participation may be discontinued if GEICAM, the investigator or the Ethical Review Board (ERB) of the study site judges it necessary for any reason.

4.3.3. Discontinuation of Study

The study may be discontinued by GEICAM if this is medically reasonable and consistent with applicable regulations of Good Clinical Practice (GCP). Stopping the study for medical reasons may be required if patients experience adverse reactions under the treatment with the study drug/medication or if new information about the safety or effectiveness of the study drug/medication justifies it.

4.4. Contraception

Patients will not be considered of childbearing potential if they are either:

- 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

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

OR

- has a congenital or acquired condition that prevents childbearing.

Patients of childbearing potential must agree to avoid becoming pregnant while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

- practice abstinence from heterosexual activity (can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception);

OR

- 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
    - 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)
        - contraceptive sponge (nulliparous women only)
        - male condom or female condom (cannot be used together)

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. Treatment

5.1. Treatments Administered

Patients will receive the following treatment:

- ✓ Pembrolizumab at a dose of 200mg as an intravenous (IV) 30 minutes infusion on day 1 of each 21-day cycle.

in combination with

- ✓ Gemcitabine at a dose of 1,250mg/m^2 as an intravenous (IV) 30 minutes infusion on day 1 and 8 of each 21-day cycle.

Treatment will be repeated on day 1 of each 21-day cycle until objective disease progression, clinical progression (under investigator criteria), unacceptable toxicity, death or withdrawal of consent, whichever occurs first. Patients completing 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication will stop pembrolizumab treatment (though may continue with gemcitabine). Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional pembrolizumab treatment if they progress after stopping it.

A lower dose of gemcitabine at 1,000mg/m^2 will be explored in combination with pembrolizumab if necessary based on the safety and tolerability of the combination as assessed in the run-in phase.

Prophylactic antiemetic therapy will be administered under site criteria.

5.2. Materials and Supplies

For this study, the term “study drug” (Investigational Medicinal Product [IMP]) refers to pembrolizumab.

Gemcitabine is “study medication” that is administered in accordance with its local prescribing information; this drug is not regarded as IMP. It is considered standard of care.

GEICAM will provide the study sites with pembrolizumab for the purpose of this study. Since gemcitabine is a marketed product approved for this indication and is administered according to the label it will not be provided by GEICAM.

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 study drug/medication in accordance with the protocol and any applicable laws and regulations.

5.2.1. Storage, preparation and administration

Investigators and site staff are reminded to continuously monitor storage temperatures and ensure that thermometers are working correctly as required for proper storage of study

drug/medication. These include thermometers for both the room storage and refrigerator storage.

Any temperature excursions related to the study drug must be reported immediately to GEICAM and documented. Once a deviation is identified, the study drug MUST be quarantined and not used until GEICAM provides documentation of permission to use the investigational product.

Study drug should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned medication should be stored separately from medication that needs to be dispensed.

Refer to the Standard Operating Procedure (SOP) at each study site for study medication storage.

5.2.1.1. Pembrolizumab

GEICAM will provide the study sites with pembrolizumab for the purpose of this study as summarized in Table 3.

Table 3. Product Descriptions

Product Name	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Infusion

Pembrolizumab should be stored at controlled refrigerator (2°C-8°C) in their original container.

Preparation and administration:

- Pembrolizumab infusion solution should be prepared in 0.9% sodium chloride (preferred diluent) or 5% dextrose and the final concentration of pembrolizumab in the infusion solution should be between 1 mg/mL and 10 mg/mL.
- Pembrolizumab solution may be stored at room temperature for a cumulative time of up to 4 hours. The 4 hour countdown begins when the vial is pierced, and includes room temperature storage of admixture solutions in the IV bags and the duration of infusion. In addition, IV bags may be stored under refrigeration at 2 °C to 8 °C for up to 20 hours. If refrigerated, allow the IV bags to come to room temperature prior to use.
- Add the required pembrolizumab into the infusion IV bag containing normal saline and gently invert the bag 10-15 times to mix the solution.
- Parenteral drug product should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if visible particles are observed.
- Do not shake or freeze the vials or the infusion solution. Caution: Do not shake the vials/bags otherwise this may result in formation of foam. If foam is noticed in either vial or

bag, the drug product will need to be discarded. A new preparation should be made, taking care not to shake or agitate the product.

- Administer the infusion solution intravenously using a sterile, non-pyrogenic, low-protein binding 0.2 to 5 μm in-line or add-on filter.
- Do not co-administer other medicinal products through the same infusion line.
- Pembrolizumab is for single use only. Discard any unused portion left in the vial.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

Pembrolizumab 200mg will be administered as a 30 minute IV infusion every 3 weeks. 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).

Drug preparation and administration will be performed at the site by a physician, registered nurse or other qualified health care provider.

5.2.1.2. Gemcitabine

Refer to the Summary of Product Characteristics (SmPC) for gemcitabine for instructions and steps necessary for drug storage, preparation and administration. Drug preparation and administration will be performed at the site by a physician, registered nurse or other qualified health care provider.

5.2.2. Accountability

It is the responsibility of the investigator to ensure that a current record of pembrolizumab and gemcitabine disposition is maintained at each study site where study drug/medication are inventoried and disposed. Records or logs must comply with applicable regulations and guidelines.

GEICAM will provide forms to facilitate inventory control if the staff at the investigational site does not have an established system that meets these requirements.

Upon completion or termination of the study, all unused and/or partially used study drug 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 local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

5.3. Method of Assigning a Patient to a Treatment

All patients who meet all criteria for enrollment will be registered to receive pembrolizumab in combination with gemcitabine.

All patients will be screened by one of the investigators prior to study entry. An explanation of the study and discussion of the expected side effects and presentation of the informed consent document will take place. Eligible and consenting patients (entered) will be enrolled into the study.

No patients can receive protocol treatment until enrollment has been performed. All eligibility criteria must be met at the time of enrollment. There will be no exceptions. Any question should be addressed with GEICAM prior to enrollment. The eligibility checklist must be completed and signed by the investigator prior to enrollment and it must be included into medical history of each patient.

The study personnel at the site will enroll to patient through the eCRF and the system will send the number of the patient registration to the site.

All eligible patients enrolled in the study will be entered in a patient registration log maintained by the GEICAM central office.

Trial treatment must be administered within 7 days from enrollment.

5.4. Special Treatment Considerations. Dose Adjustments of Study Drug/Medication

All dose modifications should be based on the worst preceding toxicity.

Every effort should be made to administer study treatment at the planned dose and schedule. However, in the event of significant treatment-related toxicity, administration of study drug/medication may need to be adjusted as described in the following sections. Depending on the nature of the toxicity observed, dose adjustments may be required for one or more study drug/medication in the combination.

All dose modifications/adjustments must be clearly documented in the patient's source notes and the appropriate section of the eCRF.

5.4.1. Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 4 below. See Section 5.6.2 for supportive care guidelines, including use of corticosteroids.

Table 4. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (i.e. diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with 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.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	

Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

[1.] Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Patients should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with GEICAM. The reason for interruption should be documented in the patient's study record.

5.4.2. Gemcitabine

Treatment can be delayed up to 12 weeks if the patient has not recovered from toxicity prior to the start of a new cycle. Treatment should be resumed if neutrophil count is > 1,500 and platelets > 100,000. In addition, for non-hematologic toxicities (with the exception of alopecia, vomiting and neurotoxicity), treatment should be delayed until resolution to Grade 1 or to the patient's baseline value before proceeding. If a patient has not recovered after 12 weeks she will be discontinued from gemcitabine (patients permanently discontinuing gemcitabine for a reason different than progression can continue with pembrolizumab).

5.4.2.1. Dose Adjustments within a Cycle

Table 5. Hematological Toxicity Day 8

Neutrophils (x 10^9/L)		Platelets (x 10^9/L)	% of total gemcitabine dose
>1.5	Y	>100	100
1-1.5	Y	>100	75
> 1.5	Y	75-100	75
1-1.5	Y	75-100	50
<1	Y/O	<75	0

Table 6. Non-hematological Toxicity Day 8

NCI CTCAE Grade	Total dose of gemcitabine
0-2 (and grade 3 nausea/vomiting)	100%
3-4 (except grade 3 nausea/vomiting)	50% or interrupted[a]

[a] This decision will depend on the toxicity observed and the investigator's criterion

If a day 8 dose is omitted for any reason it should not be subsequently administered. Doses reduced due to toxicity shall be administered as full doses in subsequent cycles unless they meet one of the criteria described in section 5.4.2.2.

5.4.2.2. Dose Adjustments for Subsequent Cycles

They will be based on the most severe toxicity experienced in the previous cycle:

Hematological Toxicity: The total dose of gemcitabine will be administered unless the patient has experienced one of the following in the previous cycle:

- Neutropenic fever,
- Prolonged Grade 4 neutropenia (more than 7 days duration),
- Thrombocytopenia with hemorrhage,
- Delay or omission of day 8 dose of the previous cycle as a result of hematologic toxicity, in which case gemcitabine will be administered at 75% of the initial dose level in subsequent cycles.

Non-hematological Toxicity: doses will be adjusted according to the following table:

Table 7. Non-hematological Toxicity

NCI CTCAE Grade	% of total dose of gemcitabine
0-2 (and grade 3 nausea/vomiting)	100
3 (except nausea/vomiting)	75
4	50 or discontinued from the study[a]

[a]This decision will depend on the type of toxicity observed and the investigator's criterion.

5.5. Medication Errors and Overdose

Medication errors may result in this study from the administration or consumption of the wrong drug, by the wrong patient, at the wrong time or at the wrong dosage strength. Such medication errors occurring to a study participant are to be captured on the AE page of the eCRFs and on the SAE form when appropriate. In the event of medication dosing error, GEICAM should be notified immediately.

Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- Medication errors involving patient exposure to the product;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating patient.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error and, if applicable, any associated AE(s) is captured on an AE eCRF page (refer to Management, Timing and Assessment of Adverse Events section for further details).

5.6. General Concomitant Medication and Supportive Care Guidelines

Patients must be instructed not to take any additional medication (over-the-counter or other products) during the study without prior consultation with the investigator. Any medications including herbal supplements, vitamins, or treatment taken by the patient from 28 days prior to the start of study treatment and up to 30 days following the last dose of investigational product and the reason for their administration must be recorded on the eCRF.

Routine postoperative care, such as dressing changes, suture removal, drain removal, or venous access (central or peripheral), does not need to be recorded. Anesthetics used for any surgical procedures performed during the patient's participation in the study can be recorded as "unspecified anesthesia" on the concomitant treatment records; it is not necessary to list the specific anesthetics. Palliative and supportive care for cancer-related symptoms will be offered to all patients in this study.

5.6.1. Prohibited Medications

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 study treatment or vaccination may be required. The investigator should discuss any questions regarding this with GEICAM. The final decision on any supportive therapy or vaccination rests with the investigator and/or the patient's primary physician.

Patients are prohibited from receiving the following therapies during the Baseline and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy (other than gemcitabine), biological or targeted therapy or endocrine therapy.
- Immunotherapy not specified in this protocol.
- Investigational agents other than pembrolizumab and gemcitabine.
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary lesion may be allowed at the investigator's discretion.
- Hormone replacement therapy, topical estrogens (including any intra-vaginal preparations), hormonal contraception, megestrol acetate, and selective estrogen-receptor modulators used with prophylactic intent are prohibited. If the patient is receiving these at the moment of entering the study, treatment should be discontinued before enrollment, or 14 days prior to enrollment in the case of oral contraception.
- 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 higher doses than physiologic doses of corticosteroids (e.g. more than oral prednisone 10 mg daily) may be approved after consultation with GEICAM except for that used for a limited time in order to prevent nausea/vomiting.

Patients 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. Patients 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.

5.6.2. Permitted Medications

Patients 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 5.4.1 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:**

  Patients 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 patients 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 [T1DM] (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 and **Grade 2-4** hypothyroidism events:
  - ✓ 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 8 below shows treatment guidelines for patients who experience an infusion reaction associated with administration of pembrolizumab.

Table 8. 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 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 pembrolizumab treatment administration.**	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). 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 pembrolizumab 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.		

5.7. Treatment Compliance

As all study treatments will be administered on an outpatient basis, it is the responsibility of the investigator to ensure that a current record of pembrolizumab and gemcitabine disposition is

maintained at each study site where study drug/medication are inventoried and disposed. Records or logs must comply with applicable regulations and guidelines.

GEICAM will provide forms to facilitate inventory control if the staff at the investigational site does not have an established system that meets these requirements.

6. Efficacy and Safety Evaluations, Sample Collection and Testing (Standard Laboratory Testing) and Appropriateness of Assessments

Study procedures and their timing (including tolerance limits for timing) are summarized in the Study Schedule, Protocol Attachment 1.

6.1. Efficacy Assessments

All assessments to be performed at baseline and during the study are specified in the Study Schedule, Protocol Attachment 1.

6.1.1. Primary Efficacy Assessments

The primary efficacy variable is Objective Response Rate (ORR).

Tumor response will be assessed using RECIST 1.1 criteria. Tumor assessment will be performed at baseline; the same method of measurement used at baseline will be used for further evaluations that will be conducted every 9 weeks. The best response across treatment will be recorded. ORR is defined as the percentage of patients with a complete or partial response out of the **Efficacy** population.

6.1.2. Secondary Efficacy Assessments

The secondary efficacy variables are:

- ✓ Clinical Benefit Rate (CBR) is defined as complete response (CR), partial response (PR), or stable disease (SD) ≥ 24 weeks according to the RECIST version 1.1.
- ✓ Response Duration (RD) is defined as the time from the first documentation of objective tumor response (CR or PR) to the first documented progressive disease using RECIST version 1.1, or to death due to any cause, whichever occurs first.
- ✓ Progression Free Survival (PFS) is defined as the time from enrollment to the first documented progressive disease, using RECIST version 1.1, or death from any cause, whichever occurs first.
- ✓ Overall Survival (OS) is defined as the time from the date of enrollment to the date of death from any cause.

6.2. Safety Assessments

Investigators are responsible for monitoring the safety of patients who have entered this study and for alerting GEICAM of any event that seems unusual.

The investigator is responsible for appropriate medical care of patients during the study.

The investigator remains responsible for following, through an appropriate health-care option, adverse events that are serious or that caused the patient to discontinue before completing the

study. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator.

During the course of the study, all patients entering the trial must be evaluated according to the schedule outlined in the flow charts and described below. The results of the evaluation will be recorded in the eCRF pages until the patients are not followed anymore.

6.2.1. Timing of Assessments

All assessments to be performed at baseline and during the study are specified in the Study Schedule, Protocol Attachment 1.

Vital signs assessments will include blood pressure, pulse and body temperature. A baseline standard 12-lead ECG is mandatory; on the other visits it will be only performed if clinically indicated.

The following safety laboratory assessments will be performed by the local laboratories, at the times specified in the Study Schedule:

- Hematology: hemoglobin, platelet count, White Blood Cell (WBC), Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC).
- Biochemistry: albumin, alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine, creatinine clearance (for patients with creatinine levels > 1.5 x ULN), glucose and urea.
- INR/PT and aPTT.
- T3 or FT3 (Total or Free triiodothyronine), FT4 and TSH
- Urinalysis

All AEs (and their relatedness to the study drug/medication) occurring during the study will be documented in the eCRF. AEs will be graded according to NCI-CTCAE version 4.0.

6.2.2. Definitions

The safety definitions are described in the table 7.

Table 9: Safety definitions

Concept	Definition
Adverse Event (AE)	Any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment.

	An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product. Laboratory abnormalities should be reported as AE only in case they lead to an action on study treatment or if they are serious.
Adverse Reaction (AR)	All untoward and unintended responses to a medicinal product related to any dose administered. All expected ARs are listed in the Investigator's Brochure (IB) in case of not authorized investigational product or Summary of Product Characteristics [SmPC] in case of an authorized investigational product. If the nature or the severity of an adverse reaction is not consistent with the applicable product information, the AR is defined as unexpected. The basis for the decision is the current version of the corresponding reference document that has been submitted and approved by the competent authority and the ethics committees. **Accountability criteria** The sponsor will classify the adverse event, based in their causation relation with the investigational product, following the Karch y Lasagna (1977) algorithm, as: o Final: there is reasonable temporal sequence between the drug administration and the existence of the adverse event. This event matches with the adverse reaction described for the investigational product, improves with the omission and reappears after its re-administration and can't be explained by other causes. o Probable: there is reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event matches with the adverse reaction described for the drug, improves with the omission and can't be explained by other causes. o Possible: there is reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event matches

	with the adverse reaction described for the drug but can be explained by other causes. o Conditional or improbable: there is reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event does not matches with the adverse reaction described for the drug and can be explained by other causes. o Not related: there is no reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event does not matches with the adverse reaction described for the drug and can be explained by other causes. For expedited reporting purposes it is considered as related the categories: final, probable and possible from Karch y Lasagna (1977) algorithm and as not related the category conditional or improbable of that algorithm. The determination of the possible relation with the study treatment is responsibility of the principal investigator of the site or the person designated by him.
Serious Adverse Event (SAE) and Serious Adverse Reaction (SAR)	Any adverse event or adverse reaction that, at any dose: o is fatal (results in death), o initial or prolonged inpatient hospitalization, o a life-threatening experience (that is, immediate risk of dying, defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe), o persistent or significant disability/incapacity, o congenital anomaly/birth defect or o an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (eg. medical,

	surgical) to prevent one of the other serious outcomes listed above. Do not confuse the concept "serious", described before, with "severe" which refers to the intensity of the AE or AR (minor/mild/severe). The following events will be considered as AEs of Special Interest (AESIs) and they have to be documented as a SAE and notified to the Pharmacovigilance Department of GEICAM immediately: pembrolizumab and/or gemcitabine overdose, misuse, abuse, medication error and cancer. Any temporary increase in the severity of a symptom or previous sickness that happens after baseline is also considered an adverse event. Pregnancies and lactations that occur from the day of study medication initiation throughout the study period up to 120 days after the last dose of trial therapy, or 30 days after the last dose of trial therapy 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.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	Any serious adverse reaction whose nature, intensity or consequences do not correspond with the reference information for the investigational product (example, Investigator Brochure [IB] in case of not authorized investigational product or Summary of Product Characteristics [SmPC] in case of an authorized investigational product). The unexpected nature of an adverse reaction is based in the fact of not being observed previously and not in what could be advanced based on the pharmacological properties of the drug.

6.2.3 Management, Timing and Assessment of Adverse Events

AE Classification	Adverse events should be classified following version 4.0 of the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute (NCI). A copy can be downloaded in the NCI web site: http://evs.nci.nih.gov/ftp1/CTCAE. The investigators team must have access to the CTCAE-NCI version 4.0. The AE not included in the CTCAE will be classified as described on Protocol Attachment 3. The causal relation between the investigational product and the AE will be assessed by the investigator using the Karch y Lasagna (1977) algorithm.
Procedure to notify an AE to GEICAM	The site must notify to GEICAM, through eCRF, the following events: o All adverse events that occur after enrollment. o Preexisting conditions that get worse during the study. o The evaluation of the possible relationship of each adverse event to the study drug/medication or protocol procedure. o The circumstances and data that causes the suspension of the treatment of a patient due to an adverse event. o The events leading to the clinical outcome of death from disease progression will be included in the efficacy analysis and are not recorded as adverse events, unless the investigator believes they could have been caused by the study drug/medication.
Timing and assessment of AE (see Protocol Attachment 4)	The site staff will report on the eCRF the information of the AE in the following periods: • **Baseline (after ICD and before study drug/medications):** study site personnel will note the occurrence and nature of each patient´s medical condition(s) and preexisting conditions in the appropriate section of the eCRF. If a patient never

	receives study drug/medication but experiences an adverse event after the ICD is signed, ONLY events the investigator believes may have been caused by a protocol procedure will be reported to GEICAM via eCRF. • **During treatment with the study drug/medications:** during the study, site personnel will record any change in the condition(s) and the occurrence and nature of any adverse events. A CTCAE grade rating will be assigned before each cycle for any adverse event experienced during the previous cycle. • **30-day post- treatment follow-up period:** each patient will have a 30-day post- treatment follow-up evaluation approximately 30 days following the discontinuation of study treatment. Patients should be closely followed for study treatment adverse events in order to detect delayed toxicity. If drug-related toxicity is present beyond 30 days post-discontinuation, patients must be followed until the toxicity resolves or improved to baseline, the relationship is reassessed as unrelated, the investigator confirms that no further improvement can be expected, another therapy is initiated, or death. • **Long-Term Follow-up Period (after the 30-day post-treatment):** only new and ongoing SAEs thought to be related to study drug/medication or protocol procedures should be documented on the eCRF and immediately reported to GEICAM via the designated transmission method, even if the study has been closed.

6.2.4. Management, Timing and Assessment of SAEs

Timing of SAEs (see Protocol Attachment 4)	All the SAEs (either spontaneously or during the trial visits) will be collected since the patient signs the Informed Consent Document (ICD). All the SAEs must be documented in the medical record of the patient and in the eCRF. A follow up of all the SAEs should be done until they are solved or until the toxicity is considered irreversible.

SAEs which do not need to be notified to the Pharmacovigilance Department of GEICAM	The following events are not considered SAEs: o A visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event). o Elective surgery planned before signing consent. o Hospitalization which is due solely to a planned study visit and without prolongation. o Routine health assessment requiring admission for baseline/trending of health status (e.g. routine colonoscopy). o Medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases. o Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative). o Progression of the malignancy during study (including signs and symptoms of progression), unless the outcome is fatal and death occurred before end of treatment. Thereafter death due to disease progression has not to be reported as SAE. o Hospitalization due to signs and symptoms of disease progression. o An overnight stay in the hospital that is only due to transportation, organization or accommodation problems and without medical background. They will be reported in the eCRF and in the patient record. The rest of SAEs must be notified as described as follows.
Procedure to notify a SAE to the Pharmacovigilance	The SAEs must be notified to the Pharmacovigilance Department of GEICAM. A member of the investigator team

Department of GEICAM	must complete and sign the GEICAM SAE notification form which will be sent by fax/mail, immediately and always during the 24 hours following knowledge of the SAE: **Pharmacovigilance Department of GEICAM** Fax: +34 917 371 619 farmacovigilancia@geicam.org GEICAM will review the received form and, if necessary, will ask more information to the investigator. When additional information is obtained about the SAEs, or this is solved or is improbable it will change, a follow-up report must be also completed and sent by fax/mail, immediately and always during the following the 24 hours to the Pharmacovigilance Department of GEICAM. If GEICAM suspects that the SAE could be a SUSAR, the investigator should give the follow up information requested. All SAEs/AESIs from the time the patient have the first dose of the study drug/medication through 30 days following the last administration of study drug/medications must be reported according to the procedure described above. All SAE regardless of timing must be reported, if considered related to study drug/medication. Likewise, progression of a patient's underlying condition leading to one of the above should also not be reported as a SAE, but documented as primary study endpoint. GEICAM will report all SAEs and AESIs immediately to the Chief Investigator. All SAEs and AESIs will be followed-up by the investigator until satisfactory resolution. Annually all SARs will be reported as the DSUR to the Competent Authorities and the leading ethics committee, including all SUSARs. Withdrawal from further treatment shall be at the discretion of the investigator.
Events of Clinical Interest (ECI)	ECI must be reported within 24 hours from the time the investigator team is aware of such an occurrence, regardless of whether or not the investigator considers the event to be related

	to study drug/medication. Events of clinical interest for this trial include: • an overdose of Pembrolizumab product, defined as any dose of 1,000 mg or greater (≥5 times the indicated dose), that is not associated with clinical symptoms or abnormal laboratory results. • an overdose of Gemcitabine product is defined as any dose that is 10% higher than the theoretical dose. • an AST or ALT ≥ 3 x ULN and an total bilirubin ≥ 2 x ULN and, at the same time, an alkaline phosphatase < 2 x ULN. In addition, these ECI require additional detailed information to be collected and entered in the study database. ECI may be identified through spontaneous patient report and / or upon review of subject data. Adverse events that are both an SAE and an ECI should be reported one time as an SAE only, however the event must be appropriately identified as an ECI as well in in the database.
Death on Study	Any death occurring during the active treatment part of the study and within 30 days following the last treatment must be reported to GEICAM as the sponsor within 24 hours, regardless of the relation to study drug/medication, and has to be reported on the death report form section of the eCRF. The cause of death should be documented (cancer-related, treatment-related, cancer- and treatment-unrelated). Autopsy reports should be collected whenever possible and sent to the GEICAM. Deaths that occur due to tumor progression do not have to be reported as a SAE unless they occurred before end of treatment. Deaths after the end of study which are considered to be related to study treatment have to be reported as SAEs. To the extent feasible sufficient information including relevant laboratory values, ECG, scan, biopsy or autopsy results must be provided by the investigator in the SAE narrative (even if investigator determines the SAE is not related) so as to permit

	an independent causality assessment by a Competent Authority.

6.2.5. Management, Timing and Assessment of SUSARs

Expedited Notification of SUSAR to the Competent Authorities/ERB	The Pharmacovigilance Department of GEICAM is responsible to notify to each of the Competent Authorities and ERBs of the participating countries, all the SUSARs collected in the study, following the procedures shown in the current legislation.
Timing of notification	The deadline for reporting a SUSAR shall be 15 calendar days from when GEICAM becomes aware of it. When suspected SUSAR caused the death of the patient or endangered her life, GEICAM will send the information within 7 calendar days from the date on which it becomes aware. This information must be completed, if possible, in the next 8 days.
Expedited reporting of other relevant safety information	GEICAM will also notify, expeditiously, all the information that could modify the balance benefit/risk of the investigational product, or determine changes in its administration pattern or in the study performance, such as: o A qualitative change or an increase in the percentage of occurrence of the SAR expected, which are considered clinically significant. o The SUSAR occurring after completion of the study and are reported by the investigator to the sponsor. o New events related to the conduct of a trial or the development of an IMP likely to affect the safety of patients, such as: ✓ SAE that could be related with the study procedure and could modify the conduct of the trial. ✓ A significant risk to patients such as lack of efficacy in a drug used to treat a life-threatening illness. ✓ A major safety finding from a newly completed animal study (such as carcinogenicity). ✓ A temporary halt of a trial for safety reasons if the trial is conducted with the same

	investigational medicinal products in another country and if this information is known by GEICAM. This relevant information shall be notified as soon as possible and no later than 15 days after GEICAM becomes aware of it. Additional information will also be notified as quickly as possible.
Annual Safety Reports	The annual safety reports that include the SAEs, SARs and SUSARs collected during the study will be sent by GEICAM to the Competent Authorities and ERBs at the time established by the current legislation.
Notification to investigators	GEICAM will communicate to the investigators any safety information that may affect the safety of trial patients, as soon as possible. Information on SUSAR occurred during the study will be sent quarterly, in aggregate, in a list along with a brief analysis of the data provided. They will be informed also, throughout the entire study, of any safety aspect that impacts the performance on the clinical trial or in the product development, including the interruption or modification in the development program of the protocol safety-related.

6.3. Other Assessments

6.3.1. Exploratory Efficacy Assessments based on immune-related (ir) RECIST

The exploratory efficacy assessments are:

- ✓ Objective Response Rate (ORR) is defined as the percentage of patients with an irCR or irPR based on irRECIST.
- ✓ Progression Free Survival (PFS) is defined as the time from enrollment to the first documented progressive disease, using irRECIST version 1.0 or death from any cause, whichever occurs first.
- ✓ Clinical Benefit Rate (CBR) is defined as irCR, irPR or irSD ≥ 24 weeks according to the irRECIST version 1.0.

- ✓ Response Duration (RD) is defined as the time from the first documentation of objective tumor response (irCR or irPR) to the first documented progressive disease using irRECIST version 1.0, or to death due to any cause, whichever occurs first.

6.3.2. Biomarker Assessments

Detailed instructions for the collection, handling and shipment of samples are outlined in the Sample Management Manual.

This trial will analyze a set of immune biomarkers in tissue and peripheral blood, in order to monitor clinical responses and correlate them with evolution of the disease and the efficacy of pembrolizumab in combination with gemcitabine, paying special attention to long term responders. This data will be compared with those from healthy volunteers (if available). An exploratory search of an immune score will be performed.

Tissue:

Time points for metastatic tumor sample collection will be at baseline, at cycle 3 (whenever possible) and at progression (whenever possible). The following biomarker analyses (included but not limited) will be performed:

* Density of intratumoral and stroma cells: TILs and subtypes (Treg, CD8+), tumor-associated macrophage (TAM) and MDSC.

* Gene expression and molecular profiling of tumors.

* Comprehensive analysis of the tumor microenvironment including:

1. RNA-seq profiling to semi-quantitatively measure transcript levels of immune related genes
2. Full exon sequencing of cancer related to genes to estimate mutational burden
3. Fluorescent in situ hybridization (FISH) to detect copy number gain of PD-L1 and PD-L2
4. PD-L1 protein expression.
5. Immunohistochemistry (IHC) for CD3 and CD8 to evaluate pattern of infiltration

Peripheral blood:

Time points for the blood assessments will be at baseline, cycle 3, and at cycle 6 or at post treatment visit, whatever occurs first. In order to analyze the immunophenotype and the expression of immune biomarkers along treatment, the following assessments will be of interest (included but not limited to the following biomarkers):

- T-cell repertoire using RNA-seq that include (but not limited to) CDR1, CDR2, and CDR3 as well as all V-D-J junctions.

- Analysis of the phenotype of peripheral blood mononuclear cells (PBMC) (e.g. CD45, CD11b, CD33, HLA-DR, CD14, CD15).
- Analysis of the cytokine profile (cytokines, interleukines and chemokines, e.g. IFN-gamma, TNF-alfa, IL2, IL4, IL6, IL7, IL8, IL10, IL13, IL17, IL21, IL23).
- Analysis of tryptophan metabolites and activity of immune checkpoints (e.g. IDO).
- Tregs Cells (e.g. lymphocytes FoxP3 positive, CD25 high, marked with CD3, CD4, CD127).
- Analysis of surface antigens (modulators of CD4+ and CD8+ lymphocytes T activity):

  A) Co-suppressors expression: e.g. CTLA4 and PD1 (labelled with CD3, CD4 and CD8).

  B) Co-stimulators expression: e.g. CD28, OX40 y HLA-DR (labelled with CD3, CD4 and CD8).

7. Data Quality Assurance

To ensure accurate, complete and reliable data, GEICAM will do the following:

- Provide instructional material to the study sites, as appropriate,
- sponsor a start-up training session to instruct the investigators and study coordinators. This session will give instructions on the protocol, the completion of the eCRFs, and study procedures,
- make periodic visits to the study site to review study progress, investigator and patient compliance with the clinical trial protocol requirements and any emergent problems,
- be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax,
- review and evaluate eCRF data and use standard computer edits to detect errors in data collection,
- conduct a quality review of the database, and
- verify the quality of the data.

To ensure the safety of participants in the study and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as original source documents for the study. If requested, the investigator will provide GEICAM, applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents.

7.1. Data Management and Registries File

Data for this study will be recorded via an electronic data capture system using eCRFs. Data will be transcribed by the site from the paper source documents onto the eCRF. In no case the eCRF is to be considered as source data for this trial. The eCRFs must be completed in an electronic Data Base. That electronic Data Base carries out with the regulatory authorities requirements. It is the responsibility of the Investigator to maintain adequate and accurate eCRFs (according to the technology used) designed by GEICAM to record (according to GEICAM instructions) all observations and other data pertinent to the clinical investigation. All eCRFs should be completed in their entirety in a neat, to ensure accurate interpretation of data.

Should a correction be made, the corrected information will be entered in the eCRF overwriting the initial information. An audit trail allows identifying the modification.

Data are available within the system to GEICAM as soon as they are entered in the eCRF.

The computerized handling of the data by GEICAM when available in the eCRF may generate additional requests to which the Investigator is obliged to respond by confirming or modifying the data questioned. The requests with their responses will be managed through the eCRF.

8. Sample Size and Statistical Methods

8.1. Determination of Sample Size

8.1.1. Sample Size determination

A Simon minimax two-stage design will be employed with the possibility of stopping early due to lack of response. Results from previous studies showed that gemcitabine produced a response rate of around 20% (46-48), this will be our expected H0. With the combination of pembrolizumab and gemcitabine, we expect to increase this rate to 35% what will be our H1 (an absolute increase of 15%), with an alpha error of 0.05 and a statistical power of 80%, we will need to include 53 evaluable patients in this trial. The first stage will include 31 evaluable patients, if at least 7 present a response, recruitment will continue to include the 53 evaluable patients. The null hypothesis of H0=20% will be rejected if 16 or more responses are observed in 53 patients.

8.2. Statistical and Analytical Plans

8.2.1. General Considerations

Statistical analysis of this study will be the responsibility of GEICAM. The interpretation of study results will be the responsibility of the chief investigator of the study.

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated and maintained by GEICAM. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

All analysis will be performed using the SAS Enterprise Guide 5.1 version.

8.2.1.1. Patient Populations

Intent to treat population (ITT): The ITT population will include all patients who are enrolled.

Efficacy population: Is a subset of the ITT population that have measurable disease, have received at least one dose of study treatment and have at least one tumor response assessment according to RECIST v.1.1 criteria (unless a progression or death or unacceptable toxicity is seen before the first tumor response assessment) and without certain major protocol deviations according to the protocol deviation manual.

The Efficacy population will be the primary population for the efficacy analysis. It will be performed a sensitivity analysis using the ITT population.

Safety population: The Safety population will include all patients who are enrolled and have received at least one dose of the study treatment.

The Safety population will be the primary population for the safety analysis.

Biomarker population: a subset of enrolled patients with available samples.

8.2.2. Patient Disposition

A detailed description of patient disposition will be provided. It will include:

- Summary of patients entered and by site.
- Total number of patients entered.
- Total number of patients enrolled.
- Summary of reasons for patients entered, but not enrolled.
- Total number of patients treated.
- Summary of reasons for patients enrolled, but not treated.

A detailed summary of reasons for patient discontinuation from study treatment will be provided.

A summary of all identified important protocol violations will be provided.

8.2.3. Patient Characteristics

Patient characteristics will include a summary of the following:

- Patient demographics.
- Baseline disease characteristics.
- Preexisting conditions/secondary conditions.
- Prior therapy.

Other patient characteristics will be summarized as deemed appropriate.

Standard descriptive statistics, such as the mean, median, range and proportion, will be used to summarize the patient sample and to estimate parameters of interest. Ninety-five percent confidence intervals will be provided for estimates of interest where possible.

8.2.4. Concomitant Therapy

A summary of concomitant therapies will be generated in the safety population.

8.2.5. Treatment Compliance

Treatment information will be collected at each dose administration. The estimate of percent compliance will be given by:

$$\text{Percent Compliance} = \frac{\text{Actual dose administered per week}}{\text{Dose expected to be administered per week}} \times 100$$

No minimal level of compliance will be defined for patient inclusion in efficacy analyses. To be considered compliant patients should have received at least 80% of the planned number of doses. Exploratory analysis of the impact of compliance on selected efficacy endpoints may be performed if deemed necessary.

8.2.6. Efficacy Analyses

All efficacy definitions are described in section 6.1.

All efficacy analysis will be based on the **Efficacy** population. Additional efficacy analyses will be performed on the ITT population.

All primary and secondary endpoints based on radiological (and photographical where applicable) assessments of tumor burden (PFS, ORR, CBR and RD) will be derived using the local radiologist's/investigator's assessment.

8.2.6.1. Analyses of Primary Endpoint

The primary endpoint is ORR.

Objective Response Rate (ORR): A patient will be considered to have achieved an objective response (OR) if the patient has a sustained complete response (CR) or partial response (PR) according to RECIST v.1.1 definitions. Otherwise, the patient will be considered as non-responders in the ORR analysis. ORR will be estimated by dividing the number of patients with objective response (CR or PR) by the **Efficacy** population ("response rate").

$$\text{Objective Response Rate} = \frac{\text{Number of CRs + PRs}}{\textbf{Efficacy} \text{ population}}$$

The ORR will be reported, including a 95% confidence interval using the Clopper-Pearson method.

These analyses will be conducted at a two-sided 0.05 level of significance.

Additional sensitivity analyses will be outlined in the SAP. For the analysis in the ITT population, patients with inadequate data for tumor assessment (e.g., no baseline assessment or no follow-up assessments) will be considered as non-responders in the OR rate analysis.

In addition, the best objective response for each patient will be summarized.

8.2.6.2. Analysis of Secondary Endpoints

The secondary efficacy endpoints are:

Progression-Free survival (PFS): PFS data will be censored on the date of the last tumor assessment on study for patients who do not have objective tumor progression and who have not died due to any cause while on study. Additionally, patients who start a new anti-cancer therapy prior to documented PD will be censored at the date of the last tumor assessment prior to the start of the new therapy.

The primary analyses of PFS will be performed in the **Efficacy** population. Additionally a similar analysis will be also performed in the ITT population.

Overall Survival (OS): OS data will be censored on the last date the patient is known to be alive.

OS will be analyzed in the **Efficacy** population. Additionally a similar analysis will be also performed in the ITT population.

Response Duration (RD): RD data will be censored on the date of the last tumor assessment on study for patients who do not have objective tumor progression and who have not died due to any cause while on study. Additionally, patients who start a new anti-cancer therapy prior to documented PD will be censored at the date of the last tumor assessment prior to the start of the new therapy.

RD will only be calculated for the subgroup of patients with an objective response.

Clinical Benefit Rate (CBR): A patient will be considered to have clinical benefit (CB) if the patient has a sustained complete response (CR) or partial response (PR), or stable disease (SD) ≥ 24 weeks according to RECIST v.1.1 definitions. Otherwise, the patient will be considered as not achieving CB in the CBR analysis. CBR on study treatment will be estimated by dividing the number of patients with CR, PR, or SD ≥ 24 weeks by the **Efficacy** population by treatment arm.

$$\text{Clinical Benefit Rate} = \frac{\text{Number of CRs + PRs + SDs} \geq \text{24 weeks}}{\textbf{Efficacy} \text{ population}}$$

The CBR will be reported, including a 95% confidence interval using the Clopper-Pearson method.

All of the above secondary analyses will be conducted at a two-sided 0.05 level of significance.

For the CBR analysis in the ITT population, patients with inadequate data for tumor assessment (e.g., no baseline assessment or no follow-up assessments) will be considered as not achieving CB in the CBR analysis.

Additional sensitivity analyses will be outlined in the SAP.

Patients without post enrollment assessments will be censored at the date of enrollment +1 day in all time to event analyses.

8.2.6.3. Analysis of Exploratory Endpoints

The exploratory efficacy assessments are:

Objective Response Rate (ORR): A patient will be considered to have achieved an objective response (OR) if the patient has a sustained irCR or irPR according to irRECIST definitions. Otherwise, the patient will be considered as non-responders in the ORR analysis.

Progression-Free survival (PFS): Progression-Free Survival (PFS) assessed according to irRECIST by the investigator.

Clinical Benefit Rate (CBR): A patient will be considered to have CB if the patient has a sustained irCR or irPR, or irSD ≥ 24 weeks (+/- 2 weeks) according to irRECIST definitions. Otherwise, the patient will be considered as not achieving CB in the CBR analysis

Response Duration (RD): Response Duration (RD) assessed according to irRECIST.

8.2.7. Safety Analyses

The toxicity and tolerability of study drug/medication will be evaluated in the safety population. Safety analyses will include summaries of the incidence of adverse events by maximum CTCAE grade (v4.0; NCI 2010) that occur during the study treatment period or within 30 days of the last dose of study treatment, regardless of causality and according to the relationship to study drug/medication as assessed by the investigator. Additionally, the following safety-related outcomes will be summarized:

- Study treatment discontinuations due to adverse events.
- Deaths.
- SAEs.
- Hospitalizations and transfusions.
- Use of key concomitant medications or growth factors.

Analyses for data with discrete dates, for example, deaths, transfusions, and concomitant medications, will be done through 30 days after each patient's last dose of study treatment. Adverse events will also be analyzed in this timeframe; that is, if an event starts within 30 days

of discontinuation from study treatment, but after 30 days after the last dose of study treatment, it will not be included.

AEs data and SAEs will be presented in frequency tables by grade. Hematological and clinical biochemistry toxicities will be assessed from laboratory test parameters. The safety analysis will be performed in the safety population.

8.2.8. Other Analyses

8.2.8.1. Biomarker Analysis

The biomarker analysis of the present study will be exploratory and primarily make use of descriptive statistical methods. For continuous variables, descriptive statistics including the mean, standard deviation, median, minimum and maximum values will be provided. Categorical variables will be summarized by numbers and proportions (if possible, the 95% confidence intervals will be calculated).

Appropriate statistical methods will be used to investigate any possible relationship of biomarker levels with the efficacy end-points and outcome and to compare with data from healthy volunteers (if available). Any additional sensitivity analyses will be outlined in the specific statistical analysis plan.

8.2.9. Subgroup Analyses

Exploratory subgroup analysis may be performed if deemed appropriate.

8.2.10. Criteria for End of Study

This study will be considered complete following the data cut-off date and datalock for the final analysis. The data cut-off date for the final analysis will occur after all enrolled patients have died or withdraw of the informed consent, or there is sufficient data to achieve the primary and secondary objectives, whatever occurs first.

If further data are collected that are not included as part of the final locked database, the postlock data will eventually be combined with the locked database and stored in a data library separate from the locked database.

The end date of study is date of last patient´s death or the date when there is sufficient data to achieve the primary and secondary objectives and all patients have ended the study treatment, whichever comes first.

Performing exploratory objectives will be independent of the date of the end of the study.

9. Informed Consent, Confidentiality, Responsibility Insurance and Regulatory Considerations

9.1. Informed Consent

The investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the study, including answering any questions the patient may have throughout the study and sharing any new information that may be relevant to the patient's willingness to continue his or her participation in the trial in a timely manner.

The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study.

The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study drug/medication.

As used in this protocol, the term "informed consent" includes all consent and assent given by patients or their legal representatives.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

9.2. Respect of Confidentiality

The investigator will be responsible for preserving the suitable information about each patient (for example, name, address, telephone number, social security number and study identification) so that the competent authorities can have access to said information if necessary. These records must be confidentially preserved for the time indicated by the legislation.

The investigators and GEICAM will maintain the confidentiality of all patients participating in the study, according to Good Clinical Practice (GCP) and local legislation.

This clinical trial will be held in accordance and in compliance with local current legislation. Any treatment of personal data that is held within the clinical trial, for Sponsor, principal investigator, center and/or any other participant in the clinical trial, especially as far as informed consent, shall conform to the provisions of Organic Law 15/1999, of December 13, Protection of Personal Data and Royal Decree 1720/2007 of 21 December, approving the Regulations implementing the approved law 15/1999, of December 13, protection of personal Data, and any other rules in the matter.

9.3. Responsibility Insurance

GEICAM has signed an insurance policy to cover the responsibilities of the investigator and those of other parties participating in the study.

9.4. Regulatory Considerations

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices and the applicable laws and regulations. The investigator, head of the medical institution, or designee will promptly submit the protocol to applicable ERB(s).

9.4.1. Investigator Information

Physicians with a specialty in medical oncology will participate as investigators in this clinical trial.

If investigators are added after the study has been approved by GEICAM, an ERB, or a regulatory agency, these additions will not be considered changes to the protocol.

9.4.2. Protocol Signatures

After reading the protocol, each principal investigator will sign the protocol signature page and send a copy of the signed page to GEICAM.

10. Practical Considerations

10.1. Monitoring, Audit and Inspections

The study will be monitored by means of regular visits of the patients. During the visits to the center, the monitor must review the original records of the patients, the records of medication stocks and document preservation. The monitor must also evaluate the study procedures and discuss the possible problems with the investigator. During the course of the study, audit visits can be carried out in the participating centers. The investigator will allow direct access to the source documents/data for the tasks of monitoring, audit, reviewed by the ERB and the inspection by the Competent Authorities.

10.2. Preservation of Study Documentation

The copies of all the relevant information will be preserved by the investigator for a period of at least 25 years after the end of the study, according to current legislation.

10.3. Protocol Modification

Once it has been authorized by the ERB and the Competent Authority any protocol modification must be documented by writing, in the form of an amendment.

The amendments must be duly identified, by its chronological order number, dated and signed by GEICAM and the Chief Investigator.

All the protocol amendments must be notified to the ERBs involved in the trial and to the Competent Authority. If the modifications are relevant, the authorization of the involved ERBs and/or the Competent Authority will be necessary before their application.

After reading the protocol amendment, each principal investigator will sign the protocol amendment signature page and send a copy of the signed page to GEICAM.

10.4. Use of the Information and Publication

All the information concerning the study treatment provided by GEICAM in relation to this study, and not previously published, is considered to be confidential information with property right of GEICAM. This information comprises the basic information about the product, the clinical protocol, the work forms where appropriate, the eCRFs, the assessment methods, the technical methodology and the basic scientific data. This confidential information will be property of GEICAM, it must not be disclosed to third parties without the prior written consent of GEICAM and it must not be used other than for the purposes of the study.

The information developed during the practice of this clinical study is also considered to be confidential. This information can be disclosed to the extent considered necessary by GEICAM.

To allow the use of the information derived from this study and to ensure the compliance with the current rules, the investigator is obliged to provide GEICAM with all the results of examinations and all the data developed in this study. Except in that required by law, the information obtained during the study can only be provided to the doctors and to the Competent Authorities by GEICAM.

GEICAM commits to comply with current legislation relating to studies, which establishes the obligation to publish the results, both positive and negative, in conferences and journals, with reference to ERB that approved the study, and its funding source. The list of authors will be developed in accordance with the GEICAM SOPs. The different disclosures will be decided by the Chief Investigator. By signing this protocol, the Chief Investigator and Principal Investigators accept the terms of the GEICAM publications SOP and commit to respect them.

10.5. Ethics Committees

The protocol and the informed consent document will be reviewed by the involved ERBs. The single decision of the ERBs referring to the development of the study will be provided in writing to GEICAM.

GEICAM will submit the required reports of the progress of the study to the ERB and will communicate the possible SAE, the life-threatening adverse events and deaths. At the end of the study, GEICAM must inform the ERB of trial closure.

11. References

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Protocol Attachment 1. Study Schedule

Study Schedule of Events and Timelines. GEICAM/2015-04 (PANGEA-Breast)			**During Study Treatment[q] (All visits +/-3 days of scheduled treatment day)**								**Post-treatment[r] 30 days (+/-5 days) from the last study drug/medication dose**	**After study treatment termination[s]**	
Cycle	**Baseline**		**Cycle 1**		**Cycle 2**		**Cycle 3**		**Subsequent Cycles**			**PFS Follow-up Period every 9 weeks (+/-7 days)**	**OS Follow-up Period every 6 months (+/-7 days)**
Day of cycle		ENROLLMENT	**1**	**8**	**1**	**8**	**1**	**8**	**1**	**8**			
Procedure/Laboratory/ Diagnostic Test	**Within 28 days**												
ICD for Entry (before any study specific tests)[a]	**X**												
Inclusion/Exclusion Criteria	**X**												
Medical and surgical history and demographics[b]	**X**												
Physical examination[c]	**X**		**X**		**X**		**X**		**X**		**X**		
ECOG PS	**X**		**X**		**X**		**X**		**X**		**X**		
Hematology[d]	**X**		**X[e]**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		
Biochemistry[f]	**X**		**X[e]**		**X**		**X**		**X**		**X**		
INR/PT and aPTT	**X**		**X[e]**		**X**		**X**		**X**		**X**		
T3 or FT3, FT4 and TSH	**X**		**X[e]**				**X**		**X[h]**		**X**		
Urinalysis[g]	**X**		**X[e]**				**X**		**X[h]**		**X**		
Pregnancy test	**X[i]**												
Standard 12-lead ECG	**X**		**If clinically indicated**										

Study Schedule of Events and Timelines. GEICAM/2015-04 (PANGEA-Breast)			**During Study Treatment[q] (All visits +/-3 days of scheduled treatment day)**								**Post-treatment[r] 30 days (+/-5 days) from the last study drug/medication dose**	**After study treatment termination[s]**	
Cycle	**Baseline**		**Cycle 1**		**Cycle 2**		**Cycle 3**		**Subsequent Cycles**			**PFS Follow-up Period every 9 weeks (+/-7 days)**	**OS Follow-up Period every 6 months (+/-7 days)**
Day of cycle			**1**	**8**	**1**	**8**	**1**	**8**	**1**	**8**			
Concomitant medications	**X**		**X**									**X**	
AEs and SAES[j]	**X**		**X**										
Pembrolizumab dosing			**X**		**X**		**X**		**X**				
Gemcitabine dosing			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			
Tumor Assessment	**X**[k]		**Every 9 weeks from the start of treatment.**									**X**[l]	
Date of death													**X**[m]
Biomarker assessment													
Tumor tissue	**X**[n]						**X**[n]					**X**[n]	
Peripheral blood			**X**[o]				**X**[p]		**X**[p]		**X**[p]		

Study Schedule of Events and Timelines. Protocol GEICAM/2015-04 (PANGEA-Breast)

a	Signed, written informed consent (approved by ERB) obtained prior to any study specific procedure.
b	Includes local laboratory ER/PgR/HER2/Ki67 expression levels and methods used to assess them, previous treatments. Sex, Race and Age.
c	Physical examination includes measurements of height (baseline only), weight, area surface body (baseline only unless patient experience a body weight variation greater than 10% during the treatment period with gemcitabine), blood pressure, pulse rate and body temperature.
d	Hemoglobin, platelet count, White Blood Cell (WBC), Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC).
e	If baseline Hematology, Biochemistry, INR/PT and aPTT, T3 or FT3, FT4 and TSH and Urinalysis is performed within 7 days prior to the first dose of study treatment, there is no need to repeat those tests on Day 1 Cycle 1.

f	Albumin, alkaline phosphatase, ALT, AST, total bilirubin serum creatinine, creatinine clearance (for patients with creatinine levels > 1.5 x ULN) glucose and urea. If necessary, creatinine clearance should be calculated per institutional standard.
g	Blood, glucose, protein, specific gravity and microscopic exam (if abnormal). Dipstick is not permitted.
h	T3 or FT3, FT4 and TSH and Urinalysis to be performed every odd cycle day 1.
i	All patients of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to first dose of study treatment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
j	After informed consent form signature, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected. Adverse events to be monitored continuously during the treatment period. All AEs occurring during the study and until the post-treatment visit 30 days after the last study medication to be recorded with grading according to NCI-CTCAE, thereafter all study drug/medication-related SAEs should continue to be collected.
k	Disease assessment for all patients at baseline will include: CT or MRI scan of the chest, abdomen and pelvis (CAP); bone scans mandatory if the patient has bone disease or if there is any suspicious of bone metastases, any suspicious abnormalities (i.e., hotspots) identified on the bone scans at baseline must be confirmed by X-ray, CT scan with bone windows or MRI, bone lesions identified at baseline will follow the same assessment schedule as for measurable lesions; brain CT or MRI scan is mandatory if there is any suspicious of CNS metastases; CT or MRI scan of any other sites of disease as clinically indicated; clinical assessment of superficial disease which will include photographs of all superficial metastatic lesions. All lesion measurements must be recorded in the eCRF. To be performed every 9 weeks from the start of treatment ± 1 week.
l	Only to be performed post-study treatment if disease progression has not yet been confirmed and patients has not begun a new therapy. The tumor assessment will be performed every 9 weeks from the last tumor assessment.
m	The patients will be followed for survival until death, loss to follow-up, withdrawal of consent or study termination by GEICAM. After progression, the tumor assessment will be performed according to the standard medical practice. The date of death and all treatments received by the patient after progression (according to the standard medical practice) will be collected in the eCRF.
n	Time points for metastatic tumor sample collection will be at baseline, at cycle 3 (whenever possible) and at progression (whenever possible).
o	The sample has to be collected pre-dose or within 7 days prior to the first dose of treatment**.**
p	Peripheral blood assessments will be performed at cycle 3 and 6 or at post treatment visit (in case of pembrolizumab discontinuation), whatever occurs first.
q	Pembrolizumab will continue until patients complete 24 months of uninterrupted treatment or 35 administrations of it (whichever is later) and may continue with gemcitabine.
r	Subjects who are eligible for retreatment with pembrolizumab (as described in Section 3.1.4) may have up to two safety follow-up visits, one after the initial pembrolizumab discontinuation (if gemcitabine is also discontinued) and one after the Second Course Phase.
s	Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 3.1.4 will move from the follow-up phase to the Second Course Phase when they experience disease progression.

Protocol Attachment 2. Eastern Cooperative Oncology Group Performance Status

ECOG Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. 1982. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5(6):649-65

Protocol Attachment 3. Adverse event (AE) non defined in CTCAE

CTC Grade	Equivalent to:	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity.
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated although this could improve the overall well-being or symptoms of the patient.
Grade 3	Severe	Inability to work or perform normal daily activity; treatment or medical intervention is indicated in treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the patient at direct risk.
Grade 4	Life-threatening / disabling	An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing daily activities; treatment or medical intervention is required in order to maintain survival.
Grade 5	Death	AE resulting in death.

Protocol Attachment 4. Adverse Events / Serious Adverse Events Assessment Guide

Time	After ICD Before Drug	During Therapy	30-Day Post- treatment Follow-up Period	Long-Term Follow-up Period
Events to Collect	AE/SAEs Related to Procedures	New/Ongoing AE/SAEs Regardless of Relatedness to Study Treatment or Procedures		New/Ongoing SAEs Related to Study Treatment or Procedures

Abbreviations: AE = adverse event, ICD = informed consent document, SAE = serious adverse event.