GEICAM
spanish breast cancer group

Statistical Analysis Plan (SAP)

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 study"

Sponsor: GEICAM (Spanish Breast Cancer Research Group Foundation)

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This Statistical Analysis Plan was created according the ICH Good Clinical Practice[CITATION ICH \l 3082][CITATION ICH1 \l 3082], GEICAM policies and Standard Operating Procedures (SOP); and is consistent with the study protocol[CITATION GEI2 \l 3082].

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ABBREVIATIONS AND DEFINITIONS

ABC	Advanced Breast Cancer
AE	Adverse Event
ANC	Absolute Neutrophil Count
aPTT	Activated Partial Thromboplastin Time
CB	Clinical Benefit
CBR	Clinical Benefit Rate
CI	Confidence Interval
CISH	Chromogenic In Situ Hybridization
CNS	Central Nervous System
CR	Complete Response
CTCAE	Common Terminology Criteria for Adverse Events
CT Scan	Computed Tomography Scan
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.
ECOG	Eastern Cooperative Oncology Group
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 Group
HER2	Human Epidermal Growth Factor Receptor 2
HIV	Human Immunodeficiency Virus

INR	International Normalized Ratio
irRC	Immuno-related Response Criteria
ITT	Intent To Treat
MDSC	Myeloid Derived Suppressor Cells
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
OR	Objective Response
ORR	Objective Response Rate
OS	Overall Survival
PD	Progressive Disease or Programmed Death depending on the context
PFS	Progression-Free Survival
PgR	Progesterone Receptor
PR	Partial Response
PT	Prothrombin Time
RD	Response Duration
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended Phase II Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SISH	Silver In Situ Hybridization
TLFs	Tables, Listings and Figures
ULN	Upper Limit of Normal

1. INTRODUCTION

The purpose of this Statistical Analysis Plan is to give a detailed description of the statistical analysis to be performed to generate the final study report for GEICAM/2015-04 study (PANGEA-Breast).

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 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 or biological profiles in peripheral blood and tumor tissue, 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. This translational research may shed light on the putative mechanisms of the eventual efficacy of this combination and ultimately identify immune biomarkers or biological profiles that may predict clinical activity.

2. STUDY 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 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.

2.3 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.

3. STUDY DESIGN

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 plain 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.

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.

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/m2 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 the 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/m2 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, and the study medical monitor and 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.

3.1 Sample Size

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%). Considering 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 have 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.

3.2 Randomization

Not applicable.

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 v1.1.
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, 1 or 2 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) and two or more prior lines of hormone therapy in hormone receptor positive disease.
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. from protocol 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. Patients 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 of the protocol.

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 patients 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. Patients 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. Patients will resume therapy at the same dose and schedule at the time of initial discontinuation.

4.3.3 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.

4.3.4 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 Intent to treat population

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

4.5 Efficacy population

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 [CITATION EAE09 \l 3082] (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.

4.6 Safety population

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

4.7 Biomarker population

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

4.8 Second course population

Second course population will include all patients who are enrolled in second course phase.

5. ENDPOINTS AND STUDY VARIABLES

5.1 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[CITATION EAE09 \l 3082].

5.2 Secondary End-points

The following secondary end-points will be studied:

- Efficacy:
    - Progression-Free Survival (PFS) assessed according to RECIST version 1.1[CITATION EAE09 \l 3082] 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[CITATION EAE09 \l 3082].
    - Response Duration (RD) assessed according to RECIST version 1.1[CITATION EAE09 \l 3082].
    - 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[CITATION USD09 \l 3082].

5.3 Exploratory End-points

The following exploratory end-points will be studied:

- Efficacy:

- Immuno-related Objective Response Rate (iORR) is defined as iCR plus iPR according to iRECIST[CITATION GEI3 \l 3082].
- Immuno-related Progression-Free Survival (iPFS) assessed according to iRECIST [CITATION GEI3 \l 3082] by the investigator.
- Immuno-related Clinical Benefit Rate (iCBR) defined as iCR plus iPR plus iSD lasting ≥ 24 weeks according to iRECIST [CITATION GEI3 \l 3082].
- Immuno-related Response Duration (iRD) assessed according to iRECIST [CITATION GEI3 \l 3082].

- Any biomarker analyzed in tumor tissue and blood samples.

6. DATA SCREENING AND ACCEPTANCE

7.1 Missing data

The frequency of missing data will be examined and reported for each variable in the analysis. We will not perform data imputation for missing data.

6.1.1 Missing date

If the day of the month is missing for any date used in a calculation, the 15th of the month will be used to replace the missing date unless the calculation results in a negative time duration (e.g., date of onset cannot be prior to day one date). In this case, the date resulting in 1 day duration will be used. If the day of the month and the month are missing for any date used in a calculation, the date will be considered missing.

6.2 Statistical software

The statistical analysis will be developed in SAS Enterprise Guide v5.1.

6.3 Database lock

The first database lock will be carried out when run-in phase have been completed and recommended phase II dose of gemcitabine have been chosen.

The database lock for efficacy analysis of phase II will be carried out when there will be sufficient data to achieve the primary and secondary objectives and all patients have ended the study treatment, whichever comes first.

7. INTERIM ANALYSIS

No interim analysis will be carried out in this study.

7.1 Purpose of interim analysis

Not applicable.

7.2 IDMC (Interim Data Monitoring Committee)

Not applicable.

7.3 Criteria for End of Study

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

If further data are collected and 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 of study date 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 end of the study date.

8. STATISTICAL METHODS AND ANALYSES

8.1 Statistical Methods

For descriptive analyses, frequencies, percentages and ninety-five percent confidence intervals of interest will be calculated for categorical variables wherever possible. For continuous variables, standard descriptive statistics, such as total number of observations, number of available data, mean, standard deviation, minimum, percentil 25, median, percentil 75 and maximum will be calculated.

For efficacy analyses the 2-sided 95% confidence interval will be presented wherever possible. A bilateral (2-sided) 5% significance level will be used to assess the statistical significance.

Chi-square χ^2 test or Fisher exact test will be used to explore the relationship between two qualitative variables. Mann-Whitney-Wilcoxon test will be used to compare a quantitative variable in two independent groups.

Logistic regression models will be used to test the association of covariables with a binary variable (ORR, CBR) and to estimate odds ratios and their 95% confidence intervals.

Kaplan-Meier Method will be used to estimate the survival function, survival median and probabilities of occurrence of event at a certain point of time.

Cox Proportional Hazards Regression Model will be used to estimate the hazard ratio and assess the association between several risk factors, considered simultaneously, and survival time (PFS, RD, OS).

8.2 Statistical Analyses

An exploratory and descriptive analysis will be developed for each variable in the study. All continuous variables will be summarized using the following descriptive statistics: n, mean, median, standard deviation, maximum and minimum and confidence interval (CI) will be performed when this information is considered relevant to describe a unique variable. The frequency and percentages of observed levels will be reported for all categorical measures. Ninety-five percent confidence intervals will be provided for estimates of interest wherever possible.

All statistical tests will be performed with a significance level of 5%, unless otherwise specified. For qualitative comparison of independent samples the Chi-squared test (or Fisher's exact test for 2x2 tables) will be used, while for the quantitative samples Mann-Whitney-U will be used. Logistic regression models will be used to test the association of covariables with objective response (yes/no) and clinical benefit (yes/no), and to estimate odds ratios and their 95% confidence intervals. The Kaplan-Meier limit-product method will be used to estimate PFS and OS. The Kaplan-Meier survival curve will be presented graphically. Median PFS and OS with the 95% confidence interval will be reported. Cox regression models will be used to estimate hazard ratios and its 95% confidence interval. The Wald test will be used to establish the prognostic importance of each covariable.

8.2.1 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.

8.2.2 Patient Characteristics

Patient characteristics will include a summary of the following:

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

8.2.3 Concomitant Therapy

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

8.2.4 Treatment Compliance

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

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

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.

8.2.5 Dose Limiting Toxicity

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.

The total of patients with a DLT in the first cycle will be presented in order to choose the recommended phase II dose of Gemcitabine. AEs and SAEs in first cycle will be reported in frequency tables by grade. It will be indicated the number and percentage of those specific adverse events qualifying for DLT, with the name and grade, per patient and in each dose level.

8.2.6 Efficacy Analyses

All efficacy analyses 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[CITATION EAE09 \l 3082] 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").

$$Objective\ Response\ Rate = \frac{Number\ of\ CRs + PRs}{Efficacy\ Population}$$

The ORR will be reported, including a 95% confidence interval using the Clopper-Pearson method [CITATION Kei08 \l 3082].

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

Additionally a similar analysis will be also performed in the ITT population as sensitivity analysis. 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 ORR 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 analysis of PFS will be performed in the **Efficacy** population. Additionally a similar analysis will be also performed in the ITT population as a sensitivity analysis.

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 as a sensitivity analysis.

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[CITATION EAE09 \l 3082] 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.

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

The CBR will be reported, including a 95% confidence interval using the Clopper-Pearson method [CITATION Kei08 \l 3082].

All of the above secondary analyses will be conducted at a two-sided 0.05 level of significance. Additionally a similar analysis will be also performed in the ITT population as a sensitivity analysis. 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.

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 clinical enpoints are:

Immuno-related **Objective Response Rate (iORR)**: A patient will be considered to have achieved an immuno-related objective response (iOR) if the patient has a sustained iCR or iPR according to iRECIST [CITATION GEI3 \l 3082] definitions. Otherwise, the patient will be considered as non-responders in the iORR analysis.

Immuno-related **Progression-Free survival (iPFS)**: Immuno-related Progression-Free Survival (iPFS) assessed according to iRECIST [CITATION GEI3 \l 3082] by the investigator.

Immuno-related **Clinical Benefit Rate (iCBR)**: A patient will be considered to have iCB if the patient has a sustained iCR or iPR, or iSD ≥ 24 weeks (+/- 2 weeks) according to iRECIST [CITATION GEI3 \l 3082] definitions. Otherwise, the patient will be considered as not achieving iCB in the iCBR analysis

Immuno-related **Response Duration (iRD)**: Immuno-related Response Duration (iRD) assessed according to iRECIST [CITATION GEI3 \l 3082].

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)[CITATION USD09 \l 3082] 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 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. Hematologic 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 Exploratory Analyses

The biomarker analyses 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 biological subtypes/profiles and biomarker levels with the efficacy end-points and outcome, and to compare with data from healthy volunteers (if available). Any exploratory biomarker analyses, including additional sensitivity analyses, will be outlined in the specific statistical analysis plan/summary.

8.2.9 Other Subgroup Analyses

Other exploratory subgroup analyses may be performed if deemed appropriate.

8.2.10 Second Course Phase Analyses

Exploratory second course phase analyses may be performed if deemed appropriate.

9. TABLES Y FIGURES

Table/Figure No.		Title of Table/Figure
1.		Recruitment of Site
2.		Consort Flowchart
	2.1	Protocol deviation
	2.2	Analysis Populations
3		Characteristics of patients
4		Study medication exposure
5		**Primary Objectives**
	5.1	Run-in phase: RP2D, TDL analysis
	5.2	Phase II: OR analysis
6		**Secondary Objectives**
	6.1	Phase II: PFS analysis
	6.2	Phase II: CB analysis
	6.3	Phase II: RD analysis
	6.4	Phase II: OS analysis
	6.5	Safety Analysis
7		**Exploratory Objectives**
	7.1	Phase II: iOR analysis by iRECIST
	7.2	Phase II: iPFS analysis by iRECIST
	7.3	Phase II: iCB analysis by iRECIST
	7.4	Phase II: iRD analysis by iRECIST
	7.5	Biomarker analysis
8		**Second Course Phase Analysis**

The numbers of tables and figures do not have to match exactly with those of the statistical report. Please see Shells Tables, Listings and Figures.

10. APPENDIX

10.1 Shell Tables, Listings and Figures

See attachment "Shell TLFs GEICAM 2015-04 (PANGEA-Breast Study).docx"

11. BIBLIOGRAFHY /REFERENCES

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