Clinical Study Protocol

A phase II study of pembrolizumab and eribulin in patients with HR-positive/HER2-negative metastatic breast cancer previously treated with anthracyclines and taxanes

KELLY study (KEytruda and EribuLin in Luminal breast cancer)

Protocol number: MedOPP127

Study drug(s):	Halaven®; Keytruda®
EudraCT#:	2016-004513-27
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SPONSOR'S SIGNATURE PAGE

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Sponsor	Signature	Signature date (DD-Mmm-YYYY)
Scientific Global Coordinator	Signature	Signature date (DD-Mmm-YYYY)

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DECLARATION OF INVESTIGATORS

Protocol Title: **A phase II study of pembrolizumab and eribulin in patients with HR-positive/HER2-negative metastatic breast cancer previously treated with anthracyclines and taxanes**

Version date: 23rd October 2019

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I have received, reviewed and understood the following:

a) Protocol: **A phase II study of pembrolizumab and eribulin in patients with HR-positive/HER2-negative metastatic breast cancer previously treated with anthracyclines and taxanes.**

b) Summary of Product Characteristics (SmPC) of eribulin and Investigator Brochure (IB) of MK3475(Pembrolizumab) containing clinical and non-clinical data on investigational product that are relevant to the study of the product on human subjects.

I have been adequately informed as to the development to date of the investigational product. I will acknowledge receipt of the updated SmPC. I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct this study as set out in this protocol.

I understand that any change made by the Investigator(s) not previously agreed by the Sponsor may constitute a protocol non-compliance, including all of the ancillary studies or procedures performed on study patients (other than those procedures needed to ensure the patients' well-being). I am aware that I can only deviate from or apply changes to the protocol without prior approval or the favorable opinion of the Ethics Committee (EC) and/or before Sponsor approval to avoid immediate risk to the trial patients. Should this occur, I agree to inform the Sponsor as to the deviation or changes in writing and their reasons, as soon as possible.

I will not begin recruiting patients to the study until I am authorized to do so by the appropriate EC and after meeting all legal and regulatory requirements of this country.

The study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and its amendments, the

International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines (ICH E6 GCP), and applicable laws and regulations.

I agree to receiving, as described in this protocol and in the ICH E6 guidelines for GCP, written informed consent from the patient or their legally authorized representative or the witnessed verbal informed consent from all patients who have been asked to participate in this study, before performing any study procedure.

I will ensure that the study drug(s) provided by the Sponsor will only be used as described in this protocol.

I am aware of the requirements for the correct reporting of serious adverse events, and I commit to document and to report such events as required by the Sponsor and in accordance with Health Authority Regulatory requirements.

I agree to provide the Sponsor or Sponsor's representative, upon request, with evidence of current laboratory accreditation, the name and address of the laboratory, and a list of normal values and ranges.

I agree to the use of study results for national and international registration, publication, and information for medical and pharmaceutical professionals.

I agree to keep all source documents and case report forms (CRF) as specified in the relevant sections of this protocol.

I agree to submit all the forms required by the Health Authorities and my updated curriculum vitae and those of the sub-Investigators and all of the members of my team (if applicable) before the start of this study, which may be submitted, in turn, to the Health Authorities.

I am aware that the Sponsor or their representative may perform audits and that the Health Authorities may carry out inspections as part of the conduct of this study. I will permit monitoring, audit and inspection and provide direct access to source data, documents and reports for these purposes.

In light of the foregoing, I hereby consent to the inclusion by the Sponsor of my contact details and professional profile in their electronic database to be used for internal purposes and for submission to Health Authorities worldwide.

Name:

_____

Signature: _____ Date: _____

Protocol synopsis

Investigational drug:	Halaven®; Keytruda®
Protocol number:	MedOPP127
Eudract Number:	2016-004513-27
Protocol Title:	A phase II study of pembrolizumab and eribulin in patients with HR-positive/HER2-negative metastatic breast cancer previously treated with anthracyclines and taxanes.
Target disease:	Hormone receptor-(HR)positive/HER2-negative metastatic breast cancer (MBC) previously treated with at least one, but not more than two, prior chemotherapeutic regimens for treatment of locally recurrent and/or metastatic disease. Prior therapy must have included an anthracycline and a taxane in any combination or order and either in the early or metastatic disease setting unless contraindicated for a given patient. Prior anti-hormonal therapy in the metastatic disease setting is mandatory.
Subjects:	Female patients age ≥ 18 years with advanced HR-positive/HER2-negative breast cancer previously treated with anthracyclines and taxanes.
Number of patients:	44 patients.
Screening criteria:	***Inclusion criteria:*** Patients will be included in the study only if they meet **ALL** of the following criteria: 1. Written informed consent prior to beginning specific protocol procedures. 2. Female patients ≥ 18 years of age. 3. Eastern Cooperative Oncology Group (ECOG) performance status must be 0 or 1 which the Investigator believes is stable at the time of screening. 4. Life expectancy ≥ 12 weeks.

	5. Patients have a histologically and/or cytologically confirmed diagnosis of breast cancer. 6. Patients have radiologic evidence of inoperable locally recurrent or MBC. 7. Patients have HER2-negative breast cancer (based on most recently analyzed biopsy) defined as a negative in situ hybridization (ISH) test or an immunohistochemistry (IHC) status of 0, 1+, or 2+ (if IHC 2+, a negative ISH test is required) by local laboratory testing. 8. Patients have HR-positive breast cancer defined as estrogen receptor (ER) and/or progesterone receptor (PR) with >1% of tumor cells positive for ER and/or PR by IHC irrespective of staining intensity. 9. Available tumor tissue for PD-L1 biomarker analysis from a newly obtained core or excisional biopsy since last progression of a metastatic tumor lesion not previously irradiated. *Note: Subjects for whom tumor biopsies cannot be obtained (e.g., inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor.* 10. Patients have measurable disease based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 as assessed by site Investigator and local radiology review. 11. Patients have received at least one, but not more than two, prior chemotherapeutic regimens for locally recurrent and/or metastatic disease. Prior therapy must have included an anthracycline and a taxane in any combination or order and either in the early or metastatic disease setting unless contraindicated for a given patient. Prior anti-hormonal therapy in the metastatic disease setting is mandatory. 12. Patients have adequate bone marrow and organ function as defined by the following laboratory values: • Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L. • Platelets ≥ 100 x 10^9/L. • Hemoglobin ≥ 9 g/dL.

	• Potassium, calcium (corrected for serum albumin), and magnesium within normal limits for the institution. • Serum creatinine ≤ 1.5 x upper limit of normal (ULN). • Alanine aminotransferase (AST) and aspartate aminotransferase (ALT) ≤ 2.5 x ULN (or ≤ 5.0 x ULN if liver metastases are present). • Total serum bilirubin within normal range (or ≤ 1.5 x ULN if liver metastases are present). Patients with known Gilbert disease who have serum bilirubin ≤ 3 x ULN may be enrolled. 13. Patients must be accessible for treatment and follow-up. ***Exclusion criteria:*** Any patient meeting **ANY** of the following criteria will be excluded from the study: 1. Patients have received previous treatment with eribulin and an/or anti-PD1 or anti-PD-L1 agents. 2. Patients have a known hypersensitivity to any of the excipients of MK3475 (pembrolizumab) or eribulin. 3. Patients who have received chemotherapy, targeted small molecule therapy, or radiotherapy within two weeks of first dose of study treatment. 4. Patients who have received monoclonal antibodies for direct antineoplastic treatment or an investigational agent/device within four weeks of first dose of study treatment. 5. Patients have known active central nervous system (CNS) metastases and/or carcinomatous meningitis. *Note: Known brain metastases are considered active, if any of the following criteria is applicable:* *a. Brain imaging during screening demonstrates progression of existing metastases and/or appearance of new lesions compared to brain imaging performed at least four weeks earlier.*

	b. Neurological symptoms attributed to brain metastases have not returned to baseline. *c. Steroids were used for brain metastases within 28 days of first dose of study treatment.* 6. Patients have peripheral neuropathy grade 2 or more. 7. Patients have a concurrent malignancy or malignancy within five years of study enrollment (with the exception of adequately treated, basal or squamous cell skin carcinoma or curatively resected cervical cancer). 8. Patients have not recovered to grade 1 or better (except alopecia) from related side effects of any prior antineoplastic therapy. 9. Patients have had a major surgical procedure within 28 days prior to starting study drug. 10. Patients have an active cardiac disease or a history of cardiac dysfunction including any of the following: • Unstable angina pectoris or documented myocardial infarction within six months prior to study entry. • Symptomatic pericarditis. • History of documented congestive heart failure (New York Heart Association functional classification III-IV). • Patients have a left ventricular ejection fraction (LVEF) < 50% as determined by multiple gated acquisition (MUGA) scan or echocardiogram (ECHO). 11. Patients have any of the following cardiac conduction abnormalities: • Ventricular arrhythmias except for benign premature ventricular contractions. • Supraventricular and nodal arrhythmias requiring a pacemaker or not controlled with medication. • Conduction abnormality requiring a pacemaker. • Other cardiac arrhythmia not controlled with medication.

	12. Uncontrolled hyper/hypothyroidism or type 1 diabetes mellitus (T1DM). Patients with hypothyroidism stable on hormone replacement will not be excluded from the trial. Patients with controlled T1DM on a stable insulin regimen may be eligible for this study. 13. Active autoimmune disease that has required systemic treatment in past 2 years (i.e., with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). *Note: Replacement therapy (e.g., thyroxine, insulin, or physiologic steroid replacement therapy (≤ 10 mg prednisone daily) for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.* 14. Prior allogenic stem cell or solid organ transplantation. 15. Active/history of pneumonitis requiring treatment with steroids or active/history of interstitial lung disease. 16. Active uncontrolled infection at the time of screening 17. Active tuberculosis. 18. Current known infection with HIV. 19. Active hepatitis B (HBV) [patients with negative hepatitis B surface antigen (HBsAg) test and a positive antibody to HBsAg (anti-HBsAg) test at screening are eligible] or hepatitis C (HCV) [patients with a positive antibody to hepatitis C (anti-HCV) are eligible only if polymerase chain reaction (PCR) is negative for virus hepatitis C RNA]. 20. Patients have any other concurrent severe and/or uncontrolled medical condition that would, in the Investigator's judgment contraindicate patient participation in the clinical study. 21. Treatment with systemic steroids (standard premedication for chemotherapy/contrast reactions, inhaled steroids, and local applications are allowed) or another immunosuppressive agent within seven days prior to study treatment initiation.

	22. Has received live vaccines within 30 days prior to first dose of study treatment. 23. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, are not allowed to participate in this study unless they are using highly effective methods of contraception during dosing and up to 120 days after study drugs discontinuation.
Study objectives:	**Primary objective:** • To assess the efficacy -as determined by the clinical benefit rate (CBR) (total number of objective responses plus stable disease for at least 24 weeks) based on RECIST v.1.1- of MK3475 (pembrolizumab) in combination with eribulin in patients with HR-positive/HER2-negative MBC who have previously received an anthracycline and a taxane (for either early or advanced disease), unless contraindicated, and between one to two lines of chemotherapy in the metastatic setting. **Secondary objectives:** • To determine the CBR based on RECIST v.1.1 in subjects with programmed death ligand-1 (PD-L1) positive tumors. • To determine the progression-free survival (PFS) based on RECIST v.1.1. • To determine the PFS based on RECIST v.1.1 in subjects with PD-L1 positive tumors. • To determine the overall survival (OS) (OS will be determined at the end of the study). • To determine the OS in subjects with PD-L1 positive tumors. • To determine the overall response rate (ORR) based on RECIST v.1.1. • To determine the ORR based on RECIST v.1.1 in subjects with PD-L1 positive tumors. • To determine the duration of response (DoR) based on RECIST v.1.1. • To determine the DoR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.

	• To assess the safety and tolerability of MK3475 (pembrolizumab) in combination with eribulin according to the US National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.3. **Exploratory objectives:** [redacted]
Type of study:	Multicenter, open-label, phase II clinical trial
Study Treatment:	All eligible patients will be treated with MK3475 (pembrolizumab) 200 mg on day 1 of each 21-day cycle and eribulin 1.23 mg/m^2 (equivalent to eribulin mesylate at 1.4 mg/m^2) on days 1 and 8 of every 21-day cycle. Treatment with MK3475 (pembrolizumab) and eribulin will continue based on physician criteria. Study follow-up will be performed 12 months after last study dose. A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons: • Completion of 35 treatments (approximately 2 years) with pembrolizumab. Please see the specific conditions at section 5.3.2 of the protocol. Note: The number of treatments is calculated starting with the first dose. Participants who stop the combination or pembrolizumab after receiving 35 doses may be eligible for retreatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 5.3.5. Participants may be retreated in the Second

	Course Phase (Retreatment) for up to an additional 17 cycles (approximately 1 year). After 35 dose administrations, patients can continue on treatment with eribulin alone.
Safety and efficacy assessments:	**Efficacy assessments:** Disease assessments [computerized tomography (CT) of the chest, abdomen, and pelvis or magnetic resonance imaging (MRI) of the abdomen and pelvis with a non-contrast CT scan of the chest in patients for whom CT scans with contrast are contraindicated] will be performed using RECIST v.1.1 every 9 weeks (± 7 days) from the first dose of study treatment up to one year (more frequently if clinically indicated). All subjects who remain on treatment for a year will subsequently have imaging performed every 12 weeks (± 7 days). Imaging should continue to be performed until radiologic evidence of disease progression, treatment discontinuation, the start of new anti-cancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first. Bone scans will also be used to assess bone metastases. If a subject has a known history of bone metastases or has new bone pain during screening, a bone scan should be obtained prior to study entry. A bone scan at follow-up is required only if they develop new or worsening symptoms or if the site believes they have attained a complete response. If a subject has no known metastatic disease in the bone or active symptoms, a bone scan at baseline is not needed. Brain imaging during the trial should be performed in subjects with known brain metastases (every nine weeks for first year, then every 12 weeks) and those with worsening and/or new neurological symptoms. After disease progression by RECIST v.1.1, if the site Investigator determines the subject is clinically stable and will benefit from continued treatment, the subject will then be managed by irRECIST. 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. **Safety assessments:** The occurrence and maximum grade of adverse events (AEs) observed throughout the study will be listed. . Any AEs that the Investigator reports as unrelated to the drug will also be reported. In this study, AEs will be assessed according to the NCI CTCAE v.4.0.3.
Endpoints	**Primary endpoint:** • CBR based on RECIST v.1.1. **Secondary endpoints:** • CBR based on RECIST v.1.1 in subjects with PD-L1 positive tumors. • PFS based on RECIST v.1.1. • PFS based on RECIST v.1.1 in subjects with PD-L1 positive tumors. • OS (OS will be determined at the end of the study). • OS in subjects with PD-L1 positive tumors. • ORR based on RECIST v.1.1. • ORR based on RECIST v.1.1 in subjects with PD-L1 positive tumors. • DoR based on RECIST v.1.1. • DoR based on RECIST v.1.1 in subjects with PD-L1 positive tumors. • Safety. **Exploratory endpoints:** [redacted]

[redacted]	[redacted]
Planned Study period:	Recruitment period is planned to be opened for 11 months. The end of study will be 12 months after last study dose in the first course of pembrolizumab phase treatment, or after the last study dose in the second course in all patients. by the Sponsor, whichever is earlier. This data point will be considered: • LPLV (Last Patient Last Visit). • End of Recruitment (LPI) (11 months): August 2018 • Last Patient Last Visit (LPLV): Dec 2020 • Final Study Report: Feb 2021

List of abbreviations

Absolute neutrophil count	ANC
Adverse event	AE
Alanine aminotransferase	ALT
Alkaline phosphatase	ALP
Anaplastic lymphoma kinase	ALK
Antibody to hepatitis B surface antigen	Anti-HBsAg
Antibody to hepatitis C virus	Anti-HCV
Aspartate aminotransferase	AST
Body surface area	BSA
Case report form	CRF
Central nervous system	CNS
Circulating tumor cell	CTC
Clinical benefit rate	CBR
Committee for Medicinal Products for Human Use	CHMP
Common terminology criteria for adverse events	CTCAE
Computerized tomography	CT
Confidence interval	CI
Cytochrome P450	CYP
Dose administration record	DAR
Duration of response	DoR
Eastern Cooperative Oncology Group	ECOG
Echocardiography	ECHO
Electrocardiogram	ECG
Electronic data capture	EDC
End of recruitment date	LPI
Epidermal growth factor receptor	EGFR

Erythropoiesis-stimulating agents	ESA
Estrogen receptor	ER
Ethics committee	EC
Event of clinical interest	ECI
Food and drug administration	FDA
False-Discovery Rate	FDR
Formalin fixed paraffin-embedded	FFPE
Gamma-glutamyl transferase	GGT
Good clinical practice	GCP
Granulocyte-colony stimulating factor	G-CSF
Health Authority	HA
Hepatitis B surface antigen	HBsAg
Hepatitis B virus	HBV
Hepatitis C virus	HCV
Hormone receptor	HR
Immune-related Response Evaluation Criteria In Solid Tumors	irRECIST
Immune-related adverse event	irAE
Immunohistochemistry	IHC
International Human Microbiome Standards	IHMS
In situ hybridization	ISH
Interferon	IFN
International Committee of Medical Journal Editors	ICMJE
International Conference on Harmonization	ICH
Intention To Treat	ITT
Intrauterine device	IUD
Intrauterine system	IUS

Intravenous	IV
Investigational medicinal product	IMP
Last patient last visit	LPLV
Left ventricular ejection fraction	LVEF
Magnetic resonance imaging	MRI
Maximum tolerated dose	MTD
Metastatic breast cancer	MBC
Multiple-gated acquisition	MUGA
National Cancer Institute	NCI
National Comprehensive Cancer Network	NCCN
Non-small cellu lung cancer	NSCLC
Non-steroidal anti-inflammatory drugs	NSAIDS
Overall response rate	ORR
Overall survival	OS
Operational Taxonomic Units	OTUs
Polymerase chain reaction	PCR
Progesterone receptor	PR
Programmed cell death 1	PD-1
Programmed cell death 1-ligand	PD-L1
Programmed cell death 2-ligand	PD-L2
Progression-free survival	PFS
Recommended phase 2 dose	RP2D
Response criteria in solid tumors	RECIST
Receiver operating characteristic	ROC
Serious adverse events	SAEs
Start recruitment date	FPI
Summary of Product Characteristics	SmPC

Thyroid-stimulating hormone	TSH
Treatment emergent adverse event	TEAE
Treatment of physician´s choice	TPC
Triple-negative breast cancer	TNBC
Tumor-infiltrating lymphocytes	TILs
Tumor proportion score	TPS
Type 1 diabetes mellitus	T1DM
Unique patient number	UPN
Upper limit of normal	ULN
US Food and Drug Administration	FDA
White blood cell	WBC

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1 STUDY BACKGROUND AND RATIONALE

1.1 Introduction

Breast cancer is the most common cancer and the second leading cause of cancer death among women worldwide. Breast cancer is a heterogeneous disease with multiple clinical presentations and tumor characteristics.

Gene expression profiling studies have classified breast tumors into a number of distinct biological and intrinsic subtypes with prognostic and therapeutic implications, thus providing a new molecular classification of breast cancer [1]. According to this classification, four main molecular subtypes have been identified: luminal A, luminal B, HER2-enriched, and basal-like. In contrast to basal-like breast cancers, which do not express estrogen receptor (ER), progesterone receptor (PR), and ER-related genes, luminal tumors express luminal cytokeratins and have the highest levels of ER expression. On the other hand, HER2-enriched tumors overexpress HER2-associated genes but do not express genes that define the luminal subtype.

To distinguish between these molecular subtypes is essential in the clinical practice. Although the best way to perform breast tumor intrinsic subtyping is to use microarrays for gene expression analysis, molecular profiling is not ready for use in clinical decision-making. Therefore, a combination of immunohistochemical surrogate markers, using ER and PR status, HER2 status, and Ki67 levels, has been validated for molecular subtyping (Table 1) [2]. However, even though there is high agreement between biomarker scoring by protein immunohistochemistry (IHC) and gene expression, the concordance is not absolute. In this way, as an example, only around 50% of HER2-enriched tumors are ER-negative/HER2-positive by IHC, and approximately 70% of ER-negative/HER2-positive tumors by IHC are classified as the HER2-enriched subtype [3].

Table 1. Tumor subtype according to St. Gallen 2013 International Expert Consensus

Tumor subtype	*Definition*
Luminal A	ER- and PR-positive HER2-negative KI67 low (< 20%) Recurrence risk ‘low’ based on multi-gene-expression assay
Luminal B	**Luminal B-like (HER2-negative)** ER-positive HER2-negative At least one of:

	PR-negative or low (< 20%) or KI67 high (≥ 20%) or Recurrence risk 'high' based on multi-gene-expression assay **Luminal B-like (HER2-positive)** ER- and HER2-positive
Basal-like	ER- and PR-negative HER2-negative
HER2-enriched	ER- and PR-negative HER2-positive

ER: Estrogen receptor; PR: Progesterone receptor.

Moreover, recent advances in breast cancer research have allowed the deconstruction of the molecular profiles of these breast cancer subtypes [4]. This has led to an increase in treatment options, including more personalized therapy and considerable improvements in patient outcomes. However, there are only a few targeted therapies approved for metastatic breast cancer (MBC), with special focus on the HER2-positive subtype, although several novel targeted therapies are currently under evaluation.

Optimal treatment for patients with MBC is dependent upon the risks and benefits associated with each treatment option, as well as with the stage of disease and performance status of each patient [5]. Anthracyclines and taxanes are frequently used as (neo) adjuvant therapy, and therefore the number of patients previously exposed to these agents by the time they develop MBC is increasing. Current available chemotherapeutic options for third-line or later treatment of MBC include the vinca alkaloids, gemcitabine, capecitabine, and ixabepilone, as well as new formulations of older drugs, such as liposomal anthracyclines and nanoparticle albumin-bound paclitaxel. Despite the large number of treatment options, until 2010 in the United States and 2011 in Europe the only approved monotherapies for late-line treatment of MBC were capecitabine and ixabepilone. Capecitabine is approved in the United States and Europe for patients who are resistant to both taxane and anthracycline regimens, and for patients who experience taxane resistance or in whom anthracycline therapy is not indicated. On the other hand, ixabepilone is only approved in the United States for use in combination with capecitabine in patients who do not respond to anthracyclines and taxanes, or as a single agent for patients who have failed on anthracyclines, taxanes, and capecitabine.

The management of MBC is complex due to the absence of clear evidence-based guidelines for clinicians and the large number of clinical studies developed with several compounds. Moreover, because consecutive diverse therapeutic regimens are administered, there is an increased risk

of different cumulative toxicities and development of drug resistance, limiting the current treatment options available. Despite these risks, overall survival (OS) in patients with MBC is increasing, and many patients with MBC still benefit from three or more lines of treatment [6]. For this reason, additional treatment options are needed for heavily pretreated MBC patients.

1.2 Antimicrotubule agents

Microtubules are polymers made from proteins called *α*- and ß-tubulin and are part of the cytoskeleton within the cytoplasm of the cell. In addition to providing structural support, microtubules take part in many other cellular processes. During the early stages of mitosis, many microtubules increase in length by attachment of more tubulin dimers to one end, and grow out from the spindle for long distances (10 □m) into the cell, searching for an unattached chromosome. If none is found, the microtubule loses dimers and shrinks again. This expansion and retraction is repeated many times until eventually it meets and becomes chemically attached to a chromosome. When every chromosome has been captured by a microtubule, they are collected into the correct order and are then separated into two halves to divide the cell in two parts. With this division, apoptosis is induced.

The central role of antimicrotubular agents in the treatment of common epithelial cancers is further highlighted by their ability to induce remission in patients with classic drug-resistant epithelial cancers. Taxanes, vinca alkaloids, and epothilones are all microtubule-targeted agents which bind to tubulin with varying affinities and target different binding sites, with subsequent disruption of microtubule dynamics [7]. This disruption occurs during mitosis with the induction of G2/M phase cell-cycle arrest that eventually leads to cell death by apoptosis. Among these agents, there are microtubule-stabilizing (paclitaxel, nab-paclitaxel, docetaxel, and the epothilones, eg, ixabepilone) and microtubule-destabilizing drugs (vinca alkaloids, eg, vincristine, vinblastine, and vinorelbine). However, current microtubule-targeted treatment is often limited by the development of drug resistance and common side effects, frequently based on high incidences of chronic peripheral sensory and motor neuropathy, with some studies reporting up to 20–30% for patients experiencing grade 3/4 neuropathic symptoms.

Eribulin mesylate (E7389) is a structurally simplified synthetic analog of the natural marine product, halichondrin B, a non-taxane microtubule dynamics inhibitor extracted from the marine sponge *Halichondria okadai* which inhibits microtubules via a novel mechanism of action [8]. Eribulin works by binding to microtubule polymerization, without affecting depolymerization, and with the additional sequestration of tubulin into nonfunctional aggregates. By inhibiting mitotic spindle formation, eribulin causes irreversible mitotic block, which leads to cell cycle arrest in the G2/M phase and apoptosis. Moreover, eribulin binds to a limited number of high affinity sites at the plus ends of the microtubules, and there is some evidence against its binding to interdimer interfaces in pre-existing polymers. This property distinguishes eribulin

mechanistically from other antimicrotubule agents, such as paclitaxel, ixabepilone, and vinblastine.

Eribulin, which retains the potency of halichondrin B against human cancer cell lines, has a mean terminal half-life of 40 hours, and minimal renal excretion have been shown in preclinical studies. Although it has been noted that this compound is metabolized by cytochrome P450 (CYP) 3A4, preclinical research established that it does not affect the metabolism of other therapeutic agents, such as diazepam, paclitaxel, midazolam, or tamoxifen, which are also metabolized by this system. Eribulin has shown antiproliferative effects against a broad range of human cancer cell lines, including breast, prostate, melanoma, and colorectal cancer, has been associated with tumor regression and elimination in a variety of well established human tumor xenograft models,and has demonstrated activity against paclitaxel-resistant cell lines, including those with mutations in ß-tubulin [9].

1.3 Clinical efficacy of eribulin

1.3.1 Phase I trials

Various phase I clinical trials have evaluated eribulin in a variety of dose regimens in patients with different types of advanced solid tumors [10, 11]. Briefly, eribulin was administered on days 1, 8, and 15 of a 28-day cycle in the weekly regimen studies and the maximum tolerated dose (MTD) was reported to be 1.4 mg/m^2 and 1 mg/m^2 (as eribulin mesylate) with further dose escalation limited by neutropenia and fatigue. On the other hand, a MTD of 2 mg/m^2 as eribulin mesylate was established on day 1 of a 21-day cycle schedule with additional dose escalation limited by neutropenia.

Interestingly, significant antitumor activity was observed in these trials with radiological responses and disease stabilizations in several tumor types, with an special interest in breast cancer patients. These data led to the initiation of phase II studies in patients with MBC, as well as in other types of solid tumors.

1.3.2 Phase II trials

Several phase II studies have assessed the antitumor activity of eribulin in heavely pretreated MBC patients. The first study (study 201) was published by Vahdat et al. who investigated the efficacy and safety of eribulin in 103 patients with MBC who had received prior treatment with an anthracycline and taxanes [12]. Based on the results of previous phase I studies, eribulin mesylate 1.4 mg/m^2 was initially administered as a 2–5-minute intravenous (IV) infusion on days 1, 8, and 15 of a 28-day cycle. After an assessment of tolerability data, which indicated that many patients were experiencing severe neutropenia on day 15, a protocol amendment was made, and a second group of patients received eribulin mesylate 1.4 mg/m^2 administered as an IV infusion over 2 to 5 minutes on days 1 and 8 of a 21-day cycle. In this study, eribulin demonstrated in the per-protocol set (n = 87) an overall response rate (ORR) by independent review of 11.5% (95% confidence

interval [CI], 5.7–20.1, all partial responses) and achieved a clinical benefit rate (CBR) of 17.2% (95%CI, 10.0–26.8). The median duration of response (DoR), median progression-free survival (PFS), and median OS were 5.6 months (95%CI, 1.4–11.9), 2.6 months (95%CI, 0.03–14.9), and 9 months (95%CI, 0.5–27.2), respectively.

In the second phase II study (study 211), reported by Cortes et al., a total of 291 patients with locally advanced disease or MBC who had received prior treatment with anthracyclines, taxanes, and capecitabine were included [13]. The patients received eribulin mesylate 1.4 mg/m^2 as a 2–5-minute IV infusion on days 1 and 8 of a 21-day cycle. In this study, eribulin showed an ORR by independent review of 9.3% (95%CI, 6.1–13.4, all partial responses) and a CBR of 17.1% (95%CI, 12.8–22.1). The median DoR, median PFS, and median OS were 4.1 months (95%CI, 1.4–11.9), 2.6 months (95%CI, 0.03–13.1), and 10.4 months (95%CI, 0.6–19.9), respectively.

Finally, in other phase II trial, reported by Aogi et al., the safety and efficacy of eribulin was investigated in 80 japanese patients with MBC who had previously been treated with an anthracycline and a taxane [14]. The study used the same dosing regimen and mode of administration as that of the study by Cortes et al. In this study, eribulin demonstrated an ORR by independent review of 21.3% (95%CI, 12.9–31.8, all partial responses) and a CBR of 27.5% (95%CI, 18.1–38.6). The median DoR, median PFS, and median OS were 3.9 months (95%CI, 2.8–4.9), 3.7 months (95%CI, range 2–4.4), and 11.1 months (95%CI, range 7.9–15.8), respectively.

1.3.3 Phase III trials

The encouraging pharmacokinetic and pharmacodynamic results observed in the phase I trials and the antitumor activity without severe adverse events (AEs) objectived in the phase II trials in patients with extensively pretreated MBC led to the design of the EMBRACE (Eisai Metastatic Breast Cancer Study Assessing Physician's Choice Versus E7389) phase III randomized trial (also known as study 305) [15]. The dosing regimen of the pivotal study [eribulin mesylate at 1.4 mg/m^2 (equivalent to eribulin at 1.23 mg/m^2) on days 1 and 8 of a 21-day cycle] was selected based on results of two completed phase II studies (study 201 and 202) which were both initiated with a dosing regimen of 1.23 mg/m^2 eribulin on days 1, 8, and 15 every 28 days. In this trial, a total of 762 patients with locally recurrent disease or MBC previously treated with 2–5 prior chemotherapy regimens (including anthracyclines and taxanes) were randomly assign (2:1 ratio) to receive eribulin or treatment of physicians' choice (TPC). Randomisation was stratified by geographical region, previous capecitabine treatment, and HER2 status. The primary endpoint was OS in the intention-to-treat set. Eribulin significantly improved median OS compared with TPC (13.1 vs. 10.6 months, HR = 0.81; p = 0.041). Median PFS was 3.7 and 2.2 months (HR = 0.87; p = 0.14), for the eribulin and TPC groups, respectively. The objective response rate was 12% in the eribulin group and 5% in the TPC group (p = 0.005). The results of the EMBRACE trial led to the approval of eribulin in the United States and Europe for the treatment of MBC in patients

who have received at least two previous chemotherapeutic regimens in the advanced setting, including an anthracycline and a taxane.

A second phase III randomized trial (study 301) compared the efficacy and safety of eribulin with capecitabine. In this trial, a total of 1,102 patients with MBC who had received prior anthracycline- and taxane-based therapy were randomly assigned (1:1 ratio) to receive eribulin or capecitabine as their first-, second-, or third-line chemotherapy for metastatic disease [16]. Stratification factors were HER2 status and geographic region. Coprimary endpoints were OS and PFS. Median OS was 15.9 and 14.5 months (HR = 0.88; p = 0.056), for the eribulin and capecitabine groups, Median PFS times for eribulin and capecitabine were 4.1 and 4.2 months, respectively (HR = 1.08; p = 0.30). Objective response rates were 11% for eribulin and 11.5% for capecitabine. Based on the results of this study, eribulin is currently indicated in Europe for the treatment of patients with locally advanced or MBC who have progressed after at least one chemotherapeutic regimen for advanced disease, including an anthracycline and a taxane in either the adjuvant or metastatic setting unless patients were not suitable for these treatments.

1.4 Clinical safety of eribulin

The most commonly reported adverse reactions related to eribulin are bone marrow suppression manifested as neutropenia, leucopenia, anemia, and thrombocytopenia. New onset or worsening of pre-existing peripheral neuropathy has also been described in patients treated with eribulin. Gastrointestinal toxicities, manifested as anorexia, nausea, vomiting, diarrhea, constipation, and stomatitis are among reported undesirable effects. Other adverse effects include fatigue, alopecia, increased liver enzymes, sepsis, and musculoskeletal pain syndrome [17].

1.4.1 Neutropenia

Neutropenia observed with eribulin was reversible and not cumulative; the mean time to nadir was 13 days and the mean time to recovery from severe neutropenia was eight days. Neutropenia was reported as a Treatment Emergent Adverse Event (TEAE) in 902/1,559 (57.9% for all grades) in the breast cancer population. However, neutropenia resulted in discontinuation in only < 1% of patients receiving eribulin.

Although the incidence is very low (< 1%), fatal cases of febrile neutropenia, neutropenic sepsis, sepsis, and septic shock have been reported with eribulin.

Severe neutropenia may be managed by the use of granulocyte-colony stimulating factors (G-CSF) or equivalent at the physician's discretion in accordance with relevant guidelines.

1.4.2 Peripheral neuropathy

In the 1,559 breast cancer patients, the most common adverse reaction resulting in treatment discontinuation with eribulin was peripheral neuropathy (3.4%). The median time to grade 2

peripheral neuropathy was 12.6 weeks. Development of grade 3 or 4 peripheral neuropathy occurred in 7.4% of breast cancer patients.

In clinical trials, patients with pre-existing neuropathy were as likely to develop new or worsening symptoms as those who entered the study without this condition. In breast cancer patients with pre-existing grade 1 or 2 peripheral neuropathy the frequency of treatment-emergent grade 3 peripheral neuropathy was 14%.

1.4.3 Hepatotoxicity

In some patients with normal/abnormal liver enzymes prior treatment with eribulin, increased levels of liver enzymes have been reported with initiation of eribulin treatment. Such elevations appeared to have occurred with eribulin treatment in the first two cycles for the majority of these patients. This hepatotoxicity is probably due to a phenomenon of adaptation to eribulin treatment by the liver and not a sign of significant liver toxicity in most patients.

1.5 Programmed cell death-1 (PD-1) checkpoint inhibition and cancer treatment

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Extensive evidence has demonstrated a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and prognosis in several tumor types.

The PD-1 receptor-ligand interaction is critical 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 excessive immune responses. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [18]. Binding of PD-L1 to PD-1 inhibits T cell activation triggered through the T cell receptor in peripheral tissues, whereas PD-L2 is thought to control immune T cell activation in lymphoid organs.

PD-L1 and PD-L2 are constitutively expressed or can be induced in a variety of cell types, and their interaction with the PD-1 receptor on tumor-specific T cells, plays a decisive role in immune evasion by tumors. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in cancer.

1.6 Preclinical studies with PD-1 immune checkpoint inhibitors

Preclinical data from mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction increases infiltration of tumor-specific CD8-positive T cells and finally guides to tumor rejection, either as a monotherapy or in combination with other treatment modalities [19, 20].

Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumor responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, breast carcinoma, acute myeloid leukemia, and colorectal carcinoma, among others. In these studies, tumor infiltration by CD8-positive T cells and increased interferon (IFN)-γ, granzyme B, and perforin expression were observed, reflecting that the principal mechanism that probably justify the antitumor activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T cell function *in vivo*.

1.7 Current approved indications for MK3475 (pembrolizumab) as per United States prescription information's

MK3475 (pembrolizumab) is a potent and highly selective humanized monoclonal antibody of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is indicated for the treatment of:

- Patients with unresectable or metastatic melanoma.
- Patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have high PD-L1 expression [tumor proportion score (TPS) ≥ 50%] as determined by a food and drug administration (FDA)-approved test, with no epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) genomic tumor aberrations, and no prior systemic chemotherapy treatment for metastatic NSCLC.
- Patients with metastatic NSCLC whose tumors express PD-L1 (TPS ≥ 1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving MK3475 (pembrolizumab).
- Patients with recurrent or metastatic head and neck squamous cell carcinoma with disease progression on or after platinum-containing chemotherapy. This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1.8 Role of immunotherapy in breast cancer

Several lines of evidence support the use of immunotherapy in breast cancer, mainly in triple-negative breast cancer (TNBC) patients, such as the heterogeinity of this tumor subtype, the presence of high PD-L1 expression levels, the role of TILs, and emerging clinical data with immune checkpoint inhibitors.

In 2011, Lehmann et al. identified six TNBC subtypes including two basal-like, a mesenchymal, a mesenchymal stem-like, a luminal androgen receptor, and an immunomodulatory subtype [21]. This immunomodulatory subtype is characterized by an elevated expression of immune genes suggesting that some patients may benefit from immune-based therapies. Moreover, PD-L1 expression is higher in breast cancer cell lines bearing a basal-like phenotype as compared with luminal subtype, and high PD-L1 expression levels have been associated with negative prognostic features such as large tumor size, high grade, ER- and PR-negative status, HER2-positive status, high proliferation, and basal and HER2-enriched subtypes [22, 23].

In addition, over recent years, a large amount of data has also revealed the importance of TILs in controlling the clinical progression of various epithelial cancers. In breast cancer, TILs are significantly more frequent in TNBC and several studies have addressed the prognostic impact of TILs in this tumor subtype in the (neo) adjuvant setting. In patients with primary TNBC, TILs have been associated with a more favorable outcome in two adjuvant phase III trials, as well as with a higher likelihood of pCR to neoadjuvant chemotherapy [24-26].

Finally, four phase I studies have evaluated the antitumor activity of MK3475 (pembrolizumab) (anti-PD-1), atezolizumab (anti-PD-L1) (alone or in combination with chemotherapy), and avelumab (anti-PD-L1) in patients with advanced TNBC.

The KEYNOTE-012 was a multicenter, non-randomized, and multicohort phase Ib trial of single-agent MK3475 (pembrolizumab). In the TNBC cohort with PD-L1 expression (≥ 1% in tumor cells), a total of 32 heavily pretreated patients were enrolled and assessed for safety and antitumor activity (58.6% of screened patients had PD-L1-positive tumors) [27]. Patients received MK3475 (pembrolizumab) at 10 mg/kg every two weeks until disease progression or unacceptable toxicity. Among the 27 patients who were evaluable for antitumor activity, ORR was 18.5%, the median time to response was 17.9 weeks (range, 7.3 to 32.4 weeks), and the median DoR has not yet been reached (range, 15.0 to ≥ 47.3 weeks).

A phase I study also assessed the efficacy of atezolizumab in TNBC patients [28]. A total of 54 extensively pretreated patients were included irrespective of PD-L1 expression (69% of patients tested positive for PD-L1 expression as defined as PD-L1 expression in ≥ 5% of immune cells). Among 21 evaluable patients, who were PD-L1-positive, ORR was 19% with a median DoR not yet reached (range, 18.0 to 56 weeks). Atezolizumab has also been evaluated in a phase I trial in combination with different chemotherapy regimens. The safety and efficacy of the combination of atezolizumab and nab-paclitaxel was assessed in a cohort of 32 patients with advanced TNBC. Confirmed ORR was 41.7% (66.7% in first-line, 25% in second-line, and 28.6% in subsequent lines) [29].

Finally, another phase I study evaluated the antitumor activity of avelumab in 168 patients with MBC refractory to standard therapy and unselected for PD-L1 expression [30]. Overall, tumor responses by Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 were

observed in eight patients for an ORR of 4.8%. The median time to response was 11.4 weeks (range, 5.7-17.7) and the median DoR was 28.7 weeks. Among TNBC patients, ORR was 8.6% (5 of 58). However, in patients with TNBC who had PD-L1-positive immune cells within the tumor, 44.4% (4 of 9) had a partial response, compared with 2.6% (1 of 39) for TNBC and PD-L1-negative immune cells.

1.9 Clinical efficacy of immune checkpoint inhibitors in ER-positive MBC

Although the presence of TILs and PD-L1 expression is less common in the luminal subtype than in TNBC, patients with ER-positive/HER2-negative tumors represent a considerable proportion of PD-L1-positive tumors given their high prevalence. Interestingly, PD-L1 expression has been detected in circulating tumor cells from patients with luminal breast cancer, and preliminary data with immune checkpoint inhibitors have been recently reported in this tumor subtype [31].

The KEYNOTE-028 was a multicenter, non-randomized, and multicohort phase Ib trial evaluating MK3475 (pembrolizumab) in patients with PD-L1–positive advanced solid tumors. The breast cancer cohort from KEYNOTE-028 included a total of 25 heavily pretreated patients with ER-positive/HER2-negative MBC with PD-L1 expression (≥ 1% in tumor cells) [32]. Patients received MK3475 (pembrolizumab) at 10 mg/kg every two weeks for up to 24 months or until disease progression or unacceptable toxicity. At a median follow-up of 7.3 months, ORR was 12% and CBR was 20%. The median time to response was 8 weeks and the median DoR has not yet been reached (range, 8.7 to > 44 weeks).

Avelumab has also been evaluated in advanced ER-positive/HER2-negative breast cancer patients in the phase I study mentioned above [30]. Of 168 patients included, 42.9% had ER-positive/HER2-negative tumors. ORR was only 2.8% in this tumor subtype.

It is not clear the reasons for why these results varied, including differences in the assays used to measure PD-L1 levels, or simply differences depending whether we are targeting the receptor, PD-1, as is done with MK3475 (pembrolizumab) , or the ligand, as is done with avelumab.

1.10 Clinical safety of immune checkpoint inhibitors

Fatigue is the most common side effect observed with immune checkpoint inhibitors, with an estimated overall frequency around 20% percent for the anti-PD-1 and anti-PD-L1 agents. However, the most relevant toxicities in clinical practice associated with these agents are immune-related AEs (irAE) [33].

1.10.1 Dermatologic toxicity

Skin toxicity is the most common irAE related to immune checkpoint inhibitors, and approximately 30-40% of patients treated with anti-PD-1 and anti-PD-L1 agents will develop dermatologic complications. This toxicity usually is the earliest irAE, with a median time to onset of 3.6 weeks after treatment initiation. Typical physical examination findings consist of a reticular and maculopapular erythematous rash on the trunk or extremities. Severe rashes such as Stevens-Johnson syndrome/toxic epidermal necrolysis have been also reported in rare cases.

1.10.2 Diarrhea/colitis

Diarrhea is a common clinical AE in patients undergoing treatment with immune checkpoint inhibitors. The onset of this toxicity occurs later than dermatologic toxicity, approximately six weeks after treatment initiation. Diarrhea of any grade has been reported in approximately 10-20% of patients treated with these agents, although grade 3 and 4 immune-mediated colitis has been seen in only 1-2% of cases. Potential risk of gastrointestinal perforation should be taken into consideration on patients with immune-related colitis.

1.10.3 Hepatotoxicity

Elevations in serum levels of the hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), have been observed with anti-PD-1 and anti-PD-L1 agents in less than 5% of patients with grade 3 and 4 events being extemely rare. Most episodes are asymptomatic laboratory abnormalities, but occasionally patients have an associated fever. Increases in total and direct bilirubin are exceptional, and usually ocurr in association with a prolonged period of AST and ALT elevations. The most frequent time of onset is around two or three months after initiation of treatment, although early or delayed events may also be seen.

1.10.4 Endocrinopathies

Inflammation of the pituitary, thyroid, or adrenal glands as a result of checkpoint blockade often presents with imprecise symptoms such as nausea, headache, fatigue, and vision changes. Although it has been difficult to establish the exact incidence of endocrinopathies associated with these compounds, clinically significant events are thought to occur in less than 5-10% of patients treated with anti-PD-1 and anti-PD-L1 agents. The most common endocrinopathies are hypophysitis and autoimmune thyroid disease, principally primary hypothyroidism secondary to a destructive thyroiditis. Hyperthyroidism associated with Graves disease and adrenal insufficiency have also been reported with these compounds.

1.10.5 Less common irAEs

Other less common immune-related toxicities have been attributed to anti-PD-1 and anti-PD-L1 agents, including:

- Nephritis (renal insufficiency, granulomatous interstitial nephritis, and lupus membranous nephropathy).
- Respiratory complications (sarcoidosis, pneumonitis, and organizing inflammatory pneumonia).
- Eye disorders (episcleritis, conjunctivitis, uveitis, or ophthalmopathy associated with Graves disease).
- Pancreatic alterations [assymptomatic elevated levels of serum amylase and lipase and type 1 diabetes mellitus (T1DM)].
- Neurologic syndromes (myasthenia gravis, Guillain-Barre syndrome, posterior reversible encephalopathy syndrome, aseptic meningitis, enteric neuropathy, and transverse myelitis).
- Hematologic disorders (red cell aplasia, neutropenia, and thrombocytopenia).

1.11 Rationale for dose selection of MK3475 (pembrolizumab) and eribulin

Although the dose of MK3475 (pembrolizumab) approved for treatment of melanoma subjects is 2 mg/kg every three weeks, and the dose of MK3475 (pembrolizumab) studied in the clinical trials KEYNOTE-012 and KEYNOTE-028 was 10 mg/kg every two weeks, the dose planned to be used in this trial is 200 mg every three weeks. This is because recent studies in other tumor types have indicated that 10 mg/kg every two weeks and 200 mg every three weeks are likely to be similar with regard to efficacy and tolerability.

Accordingly, this flat dose is also being used in the phase Ib/II study that is evaluating the safety and antitumor activity of MK3475 (pembrolizumab) and eribulin in 95 patients with advanced TNBC refractory to standard therapy and unselected for PD-L1 expression (NCT02513472). Preliminary data from the first 39 patients evaluable for efficacy in this trial have been recently reported. No dose-limiting toxicities were observed in phase 1b [34]. The recommended phase II dose (RP2D) of this combination was defined at eribulin 1.23 mg/m^2 on days 1 and 8 of every 21-day cycle and MK3475 (pembrolizumab) 200 mg on day 1 of each 21-day cycle. Among the evaluable analysis set, ORR was 33.3% and CBR was 41%, with no significant differences according to PD-L1 status. AEs observed with this combination were comparable to those reported historically with either treatment as monotherapy.

1.12 Study rationale

Significant antitumor activity of anti-PD-1 and anti-PD-L1 agents, alone and in combination with chemotherapy, has been reported in patients with advanced TNBC.

Preliminary data with MK3475 (pembrolizumab) in refractory ER-positive/HER2-negative and PD-L1-positive MBC patients have shown promising antitumor activity, resulting in response rates practically similar to those observed with the best chemotherapy agents used in this setting. However, no data are available so far with anti-PD-1 and anti-PD-L1 agents in combination with chemotherapy in this tumor subtype.

In order to determine if immunotherapy in combination with chemotherapy could benefit this population, this multicenter, open-label, phase II clinical trial will evaluate the efficacy and safety of the combination of MK3475 (pembrolizumab) and eribulin in hormone receptor (HR)-positive/HER2-negative MBC patients previously treated with anthracyclines and taxanes.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary objective

To assess the efficacy -as determined by the CBR (total number of objective responses plus stable disease for at least 24 weeks) based on RECIST v.1.1- of MK3475 (pembrolizumab) in combination with eribulin in patients with HR-positive/HER2-negative MBC who have previously received an anthracycline and a taxane (for either early or advanced disease), unless contraindicated, and between one to two lines of chemotherapy in the metastatic setting.

2.2 Primary endpoint

CBR based on RECIST v.1.1.

2.3 Secondary objectives

- To determine the CBR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- To determine the PFS based on RECIST v.1.1.
- To determine the PFS based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- To determine the OS (OS will be collected at the end of the study).
- To determine the OS in subjects with PD-L1 positive tumors.
- To determine the ORR based on RECIST v.1.1.
- To determine the ORR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.

- To determine the DoR based on RECIST v.1.1.
- To determine the DoR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- To assess the safety and tolerability of MK3475 (pembrolizumab) in combination with eribulin according to the US National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.3.

2.4 Secondary endpoints

- CBR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- PFS based on RECIST v.1.1.
- PFS based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- OS (OS will be collected at the end of the study).
- OS in subjects with PD-L1 positive tumors.
- ORR based on RECIST v.1.1.
- ORR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- DoR based on RECIST v.1.1.
- DoR based on RECIST v.1.1 in subjects with PD-L1 positive tumors.
- Safety.

2.5 Exploratory objectives

[redacted]

2.6 Exploratory endpoints

- [redacted]
- [redacted]
- [redacted]

3 STUDY DESIGN

3.1 Description of study design

This is a multicenter, open-label, phase II clinical trial to evaluate the efficacy and safety of the combination of MK3475 (pembrolizumab) and eribulin in HR-positive/HER2-negative MBC patients previously treated with anthracyclines and taxanes.

The study population consists of female patients age ≥ 18 years with advanced HR-positive/HER2-negative breast cancer who have received at least one, but not more than two, prior chemotherapeutic regimens for treatment of locally recurrent and/or metastatic disease. Prior therapy must have included an anthracycline and a taxane in any combination or order and either in the early or metastatic disease setting unless contraindicated for a given patient. Previous anti-hormonal therapy in the metastatic disease setting is mandatory.

In the absence of disease progression or unacceptable toxicity, treatment with MK3475 (pembrolizumab) and eribulin will continue based on physician criteria. MK3475 (pembrolizumab) and eribulin may be discontinued for toxicity independently of each other in the absence of disease progression.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- Completion of 35 treatments (approximately 2 years) with pembrolizumab. Please see the specific conditions at section 5.3.5.

Note: The number of treatments is calculated starting with the first dose. Participants who stop the combination or pembrolizumab after receiving 35 doses may be eligible for retreatment if they progress after stopping study treatment provided they meet the requirements detailed in section 5.3.5. Participants may be retreated in the Second Course Phase (Retreatment) for up to an additional 17 cycles (approximately 1 year).
After 35 dose administrations, patients can continue on treatment with eribulin alone.

Tumor assessments per RECIST v.1.1 (see **Appendix 2**) and irRECIST (see **Appendix 3**) will be performed approximately every nine weeks (± 7 days) for the first 12 months and every 12

weeks (± 7 days) thereafter until disease progression, treatment discontinuation, the start of new anti-cancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first. Tumor assessments will be performed on the specified schedule regardless of treatment delays.

For estimation of ORR, CBR, DoR, PFS, and OS, tumor response will be based on RECIST v.1.1 (see **Appendix 2**). In patients who continue treatment beyond radiographic disease progression per RECIST v.1.1, tumor response (ORR and PFS) may continue to be assessed using irRECIST criteria (see **Appendix 3**) until study treatment discontinuation.

Safety assessments will include the incidence, nature, and severity of AEs and laboratory abnormalities graded per the NCI CTCAE v.4.0.3. Laboratory safety assessments will include the regular monitoring of hematology, blood chemistry, coagulation, pregnancy test, and thyroid function testing. A schedule of assessments is provided in **Appendix 1**.

3.2 PD-L1 status determination

Tumor lesion will be retrospectively evaluated at a central laboratory for PD-L1 expression by IHC from a newly obtained core or excisional biopsy of a metastatic tumor lesion not previously irradiated (highly recommended). An archived metastatic tumor specimen may be submitted only upon agreement from the Sponsor in patients for whom tumor biopsies cannot be obtained.

PD-L1 positivity will be defined as PD-L1 expression in ≥ 1% tumor cells or in stroma for the purposes of this study.

3.3 Study schedule summary

The study will consist of a 28-days screening phase, a treatment phase, and a post-treatment phase (end of treatment visit and end of study) that includes safety, efficacy, and survival follow-up.

3.3.1 Screening phase

During this phase, subject eligibility is determined, including the documentation of baseline characteristics. This phase of the study will begin once the informed consent is signed by the patient and the procedures to be performed are described in **Section 6** of the protocol.

One re-screening is allowed in patients that are screening failure in this study. Patient has to re-consent Informed Consent Form (ICF) before any study procedure is done.

3.3.2 Treatment phase

Patients will receive study treatment according to the protocol and will be discontinued if one of the following situations arises:

- AEs which, according to the protocol or in the opinion of the Investigator, can cause serious or permanent damage or which rule out further treatment with the study drug.
- Disease progression is confirmed radiologically and unequivocally assesed according to the RECIST criteria version 1.1 and/or irRECIST.
- Major study protocol non-compliance.
- Patient's withdrawal from the study.
- Death.
- Study is canceled by the Sponsor.
- Completion of 35 treatments (approximately 2 years) with pembrolizumab. Please see section 5.3.5 of the protocol for the specific conditions.

Note: The number of treatments is calculated starting with the first dose. Participants who stop the combination or pembrolizumab after receiving 35 doses may be eligible for retreatment if they progress after stopping study treatment provided they meet the requirements detailed in section 5.3.5. Participants may be retreated in the Second Course Phase (Retreatment) for up to an additional 17 cycles (approximately 1 year).
After 35 dose administrations, patients can continue on treatment with eribulin alone.

3.3.3 End of treatment visit

End of treatment visit is within 30 days after the last dose of study treatment.

3.3.4 End of study

The end of study visit will be 12 months after last study dose in the first course of pembrolizumab phase treatment, or after the last study dose in the second course in all patients.
During this visit, status survival and post study anti-cancer therapy evaluation will be collected (telephone contact is acceptable).

LPLV: 12 months after last study dose or progressive disease experienced in all patients or when the trial is terminated by the Sponsor, whichever is earlier.

3.3.5 [redacted]

[redacted]

[redacted]

4 PATIENT SELECTION

The following eligibility criteria can be used in the screening of patients for whom the protocol treatment is deemed suitable. In order to determine whether this protocol is suitable for a given patient, all medical and non-medical criteria should be taken into consideration.

4.1 Study population

Female patients age ≥ 18 years with advanced HR-positive/HER2-negative breast cancer previously treated with anthracyclines and taxanes.

The patient's signed informed consent should be obtained before any trial related activities and according to local guidelines.

4.2 Inclusion criteria

Patient eligibility will be reviewed and documented by a suitable member of the Investigator's study team before the patients are enrolled in the study.

Patients must meet **ALL** the following inclusion criteria to be enrolled in the study:

1. Written informed consent prior to beginning specific protocol procedures.
2. Female patients ≥ 18 years of age.
3. Eastern Cooperative Oncology Group (ECOG) performance status must be 0 or 1 which the Investigator believes is stable at the time of screening.
4. Life expectancy ≥ 12 weeks.
5. Patients have a histologically and/or cytologically confirmed diagnosis of breast cancer.
6. Patients have radiologic evidence of inoperable locally recurrent or MBC.
7. Patients have HER2-negative breast cancer (based on most recently analyzed biopsy) defined as a negative in situ hybridization (ISH) test or an IHC status of 0, 1+, or 2+ (if IHC 2+, a negative ISH test is required) by local laboratory testing.
8. Patients have HR-positive breast cancer defined as ER and/or PR with >1% of tumor cells positive for ER and/or PR by IHC irrespective of staining intensity.

9. Available tumor tissue for PD-L1 biomarker analysis from a newly obtained core or excisional biopsy since last progression of a metastatic tumor lesion not previously irradiated.
   Note: Subjects for whom tumor biopsies cannot be obtained (e.g., inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor.
10. Patients have measurable disease based on RECIST v.1.1 as assessed by site Investigator and local radiology review.
11. Patients have received at least one, but not more than two, prior chemotherapeutic regimens for locally recurrent and/or metastatic disease. Prior therapy must have included an anthracycline and a taxane in any combination or order and either in the early or metastatic disease setting unless contraindicated for a given patient. Prior anti-hormonal therapy in the metastatic disease setting is mandatory.
12. Patients have adequate bone marrow and organ function as defined by the following laboratory values:
    - Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L.
    - Platelets ≥ 100 x 10^9/L.
    - Hemoglobin ≥ 9 g/dL.
    - Potassium, calcium (corrected for serum albumin), and magnesium within normal limits for the institution.
    - Serum creatinine ≤ 1.5 x upper limit of normal (ULN).
    - AST and ALT ≤ 2.5 x ULN (or ≤ 5.0 x ULN if liver metastases are present).
    - Total serum bilirubin within normal range (or ≤ 1.5 x ULN if liver metastases are present). Patients with known Gilbert disease who have serum bilirubin ≤ 3 x ULN may be enrolled.
13. Patients must be accessible for treatment and follow-up.

4.3 Exclusion criteria

Any patient meeting **ANY** of the following criteria will be excluded from the study:

1. Patients have received previous treatment with eribulin and an/or anti-PD1 or anti-PD-L1 agents.
2. Patients have a known hypersensitivity to any of the excipients of MK3475 (pembrolizumab) or eribulin.
3. Patients who have received chemotherapy, targeted small molecule therapy, or radiotherapy within two weeks of first dose of study treatment.

4. Patients who have received monoclonal antibodies for direct antineoplastic treatment or an investigational agent/device within four weeks of first dose of study treatment.
5. Patients have known active central nervous system (CNS) metastases and/or carcinomatous meningitis.

   Note: Known brain metastases are considered active, if any of the following criteria is applicable:

   a. *Brain imaging during screening demonstrates progression of existing metastases and/or appearance of new lesions compared to brain imaging performed at least four weeks earlier.*
   b. *Neurological symptoms attributed to brain metastases have not returned to baseline.*
   c. *Steroids were used for brain metastases within 28 days of first dose of study treatment.*
6. Patients have peripheral neuropathy grade 2 or more.
7. Patients have a concurrent malignancy or malignancy within five years of study inclusion (with the exception of adequately treated basal or squamous cell skin carcinoma or curatively resected cervical cancer).
8. Patients have not recovered to grade 1 or better (except alopecia) from related side effects of any prior antineoplastic therapy.
9. Patients have had a major surgical procedure within 28 days prior to starting study drug.
10. Patients have an active cardiac disease or a history of cardiac dysfunction including any of the following:

    - Unstable angina pectoris or documented myocardial infarction within six months prior to study entry.
    - Symptomatic pericarditis.
    - History of documented congestive heart failure (New York Heart Association functional classification III-IV).
    - Patients have a left ventricular ejection fraction (LVEF) < 50% as determined by multiple gated acquisition (MUGA) scan or echocardiogram (ECHO).
11. Patients have any of the following cardiac conduction abnormalities:

    - Ventricular arrhythmias except for benign premature ventricular contractions.
    - Supraventricular and nodal arrhythmias requiring a pacemaker or not controlled with medication.
    - Conduction abnormality requiring a pacemaker.
    - Other cardiac arrhythmia not controlled with medication.

12. Uncontrolled hyper/hypothyroidism or T1DM. Patients with hypothyroidism stable on hormone replacement will not be excluded from the trial. Patients with controlled T1DM on a stable insulin regimen may be eligible for this study.
13. 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).

    Note: Replacement therapy (e.g., thyroxine, insulin, or physiologic steroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
14. Prior allogenic stem cell or solid organ transplantation.
15. Active/history of pneumonitis requiring treatment with steroids or active/history of interstitial lung disease.
16. Active uncontrolled infection at the time of screening.
17. Active tuberculosis.
18. Current known infection with HIV.
19. Active hepatitis B (HBV) [patients with negative hepatitis B surface antigen (HBsAg) test and a positive antibody to HBsAg (anti-HBsAg) test at screening are eligible] or hepatitis C (HCV) [patients with a positive antibody to hepatitis C (anti-HCV) are eligible only if polymerase chain reaction (PCR) is negative for virus hepatitis C RNA].
20. Patients have any other concurrent severe and/or uncontrolled medical condition that would, in the Investigator's judgment contraindicate patient participation in the clinical study.
21. Treatment with systemic steroids (standard premedication for chemotherapy/contrast reactions and local applications are allowed) or another immunosuppressive agent within seven daysprior to study treatment initiation.
22. Has received live vaccines within 30 days prior to first dose of study treatment.
23. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, are not allowed to participate in this study unless they are using highly effective methods of contraception during dosing and up to 120 daysafter study drugs discontinuation.

5 TREATMENT

5.1 Formulation, packaging, and handling

Study drug packaging will be overseen by the Sponsor's Clinical Trial Supplies department and bear a label with the identification required by local law, the protocol number, drug identification,

and dosage. The packaging and labeling of the study drug will be in accordance with Sponsor standards and local regulations. Local packaging in some countries may be different.

The study drug must be stored according to the details on the Product Information. The drug label indicates the storage temperature. Upon arrival of investigational products at the site, site personnel should check them for damage, verify proper identity, quantity, integrity of seals, and temperature conditions, and report any deviations or product complaints upon discovery.

5.1.1 Eribulin

Eribulin will be supplied as a clear and colourless aqueous solution for injection provided in glass vials containing 2 ml.. Each 2 ml vial contains eribulin mesylate equivalent to 0.88 mg eribulin. The other ingredients are ethanol and water for injections, with hydrochloric acid and sodium hydroxide possibly present in very small amounts.

5.1.2 MK3475 (pembrolizumab)

MK3475 (pembrolizumab) will be supplied directly as a solution for infusion in a single-use vial.

MK3475 (pembrolizumab) solution for infusion is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution that requires dilution for IV infusion. Each vial contains 100 mg of MK3475 (pembrolizumab) in 4 ml of solution. Each 1 ml of solution contains 25 mg of MK3475 (pembrolizumab) and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

5.2 Dosage and administration

The medicinal products should be administered sequentially on day 1 of each cycle (every three weeks) as follows: MK3475 (pembrolizumab) first followed immediately by eribulin. Eribulin will also be administered alone on day 8 of each cycle.

5.2.1 Eribulin

The recommended dose of eribulin as the ready to use solution is 1.23 mg/m^2 (equivalent to eribulin mesylate at 1.4 mg/m^2) which should be administered intravenously over 2 to 5 minutes on days 1 and 8 of every 21-day cycle.

Please note: In the European Union the recommended dose refers to the base of the active substance (eribulin). Calculation of the individual dose to be administered to a patient must be based on the strength of the ready to use solution that contains 0.44 mg/ml eribulin and the dose recommendation of 1.23 mg/m^2. The dose reduction recommendations shown below are also shown as the dose of eribulin to be administered based on the strength of the ready to use solution. In the pivotal trials, the corresponding publications and in some other regions e.g., the

United States and Switzerland, the recommended dose is based on the salt form (eribulin mesylate).

5.2.2 MK3475 (pembrolizumab)

The recommended dose of MK3475 (pembrolizumab) is 200 mg administered as a 30-minute IV infusion on day 1 of each 21-day cycle. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. A window of -5 minutes/+10 minutes is allowed.

5.3 Treatment modification

Safety and tolerability of all patients will be closely monitored throughout study treatment and the follow-up period using the NCI CTCAE v.4.0.3. Patients will be assessed in order to detect any AEs before administering new study treatment during each treatment visit. Treatment will only be administered if clinical evaluation and local laboratory test results are acceptable.

If, in the opinion of the Investigator, a toxicity is considered to be due solely to one component of the study treatment (e.g., MK3475 (pembrolizumab) or eribulin) and the dose of that component is delayed or modified in accordance with the guidelines below, the other component may be administered if there is no contraindication. When treatment is temporarily interrupted because of toxicity caused by MK3475 (pembrolizumab) or eribulin, the treatment cycles will be restarted such that the MK3475 (pembrolizumab) and eribulin infusions remain synchronized. Patients who have both treatments withheld for more than 21 days must discontinue study treatment and are considered off study. Patients whose treatment is interrupted or permanently discontinued due to an AE, including abnormal laboratory value, must be followed until resolution or stabilization of the event, whichever comes first, which includes all study assessments appropriate to monitor the event.

5.3.1 Modification of the amount of study drug administered due to changes in patient's weight

MK3475 (pembrolizumab) is administered as a fixed dose irrespective of the patient´s body weight.

The amount of eribulin is calculated according to the patient's body surface area (BSA). Weight and height should be recorded at baseline and the BSA calculated, thereafter at every scheduled visit for all patients should be re-weighed. The amount to be administered must be recalculated if the patient's body weight has changed by > 10% (increased or decreased) from baseline. Re-calculation based upon smaller changes in body weight or BSA are at Investigators' discretion.

5.3.2 Eribulin

Dose delay: The administration of eribulin should be delayed on day 1 or day 8 of each cycle for any of the following:

- ANC < 1 x 10^9/L complicated or not by fever or infection.
- Platelets < 75 x 10^9/L.
- Grade 3 or 4 non-hematologic toxicities.

Retreatment following treatment interruption for toxicity may not occur until all of the following parameters have been met:

- ANC ≥ 1 x 10^9/L and no fever.
- Platelets ≥ 75 x 10^9/L.
- Grade 3 or higher non-hematologic AEs have recovered to grade ≤ 1 or baseline (or, at the Investigator's discretion, grade ≤ 2 if not considered a safety risk for the patient).

Dose reduction: Dose reduction recommendations for eribulin retreatment are shown in the following tables.

Table 2. Eribulin dose adjustments for hematologic AEs

Adverse reaction after previous eribulin administration	**Recommended dose of eribulin**
ANC < 0.5 x 10^9/L lasting more than 7 days	0.97 mg/m^2
ANC < 1 x 10^9/L neutropenia complicated by fever or infection	
Platelets < 25 x 10^9/L	
Platelets < 50 x 10^9/L and complicated by haemorrhage or requiring platelet transfusion	
Reoccurrence of any hematologic adverse reactions as specified above	
Despite dose reduction to 0.97 mg/m^2	0.62 mg/m^2
Despite dose reduction to 0.62 mg/m^2	Consider

Absolute neutrophil count: ANC.

Table 3. Eribulin dose adjustments for non-hematologic AEs

Adverse reaction after previous eribulin administration	**Recommended dose of eribulin**
Any grade 3 or 4 in the previous cycle	0.97 mg/m^2
Reoccurrence of any non-hematologic adverse reactions as specified above	
Despite dose reduction to 0.97 mg/m^2	0.62 mg/m^2
Despite dose reduction to 0.62 mg/m^2	Consider discontinuation

Do not re-increase the dose of eribulin after it has been reduced.

5.3.3 MK3475 (pembrolizumab)

AEs (both non-serious and serious) associated with MK3475 (pembrolizumab) exposure may represent an immunologic etiology. These AEs may occur shortly after study treatment initiation or several months after the last dose of treatment. MK3475 (pembrolizumab) must be withheld

for certain drug-related toxicities and severe or life-threatening AEs as per Table 4 below. Dose reduction of MK3475 (pembrolizumab) is not permitted.

MK3475 (pembrolizumab) should be permanently discontinued:

- For grade 4 toxicity except for endocrinopathies that are controlled with replacement hormones.
- If steroid dosing cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
- If a treatment-related toxicity does not resolve to grade 0-1 within 12 weeks after last dose of MK3475 (pembrolizumab) .
- If any event occurs a second time at grade ≥ 3 severity (also for recurrent grade 2 pneumonitis).

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 (CTCAEv 4.0)**	**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
	Grade 3 or 4, or	Permanently		

	recurrent Grade 2	discontinue		and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
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 (ie, 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	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		

dysfunction		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).

5.3.4 Supportive care guidelines for MK3475 (pembrolizumab)-related AEs

Pneumonitis: Steroids should be administered for grade ≥ 2 events (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper continued over no less than four weeks). MK3475 (pembrolizumab) should be withheld for grade 2 pneumonitis, and permanently discontinued for grade 3, grade 4, or recurrent grade 2 pneumonitis. Consider adding prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

Diarrhea/colitis: Subjects should be carefully monitored for signs and symptoms of enterocolitis such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever. The potential risk of gastrointestinal perforation should be taken into consideration.

All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

Steroids should be administered for grade ≥ 2 events (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper continued over no less than four weeks). MK3475 (pembrolizumab) should be withheld for grade 2 or 3 colitis, and permanently discontinued for grade 4 colitis.

T1DM (if new onset) or hyperglycemia: Insulin should be administered for T1DM and hyperglycemia, and MK3475 (pembrolizumab) should be withheld in cases of grade 3 or 4 hyperglycaemia until metabolic control is achieved. MK3475 (pembrolizumab) will be resumed when subjects are clinically and metabolically stable.

Hypophysitis: Steroids should be administered for grade ≥ 2 events (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper continued over no less than four weeks). Replacement of appropriate hormones should be administered as clinically indicated, and MK3475 (pembrolizumab) should be withheld for symptomatic hypophysitis until the event is controlled with hormone replacement.

Hepatotoxicity: Steroids should be administered [initial dose of 0.5-1 mg/kg/day (for grade 2 events) and 1-2 mg/kg/day (for grade 3 or 4 events) prednisone or equivalent followed by a taper continued over no less than four weeks]. MK3475 (pembrolizumab) should be withheld for grade

2 immune-related hepatitis, and permanently discontinued for grade 3 or 4 immune-related hepatitis.

Renal failure or nephritis: Steroids should be administered for grade ≥ 2 events (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper continued over no less than four weeks). MK3475 (pembrolizumab) should be withheld for grade 2 immune-related nephritis, and permanently discontinued for grade 3 or 4 immune-related nephritis.

Hyperthyroidism or hypothyroidism: Hypothyroidism may be managed with replacement therapy without treatment interruption and without steroids. Hyperthyroidism must be managed symptomatically and steroids should be administered for grade ≥ 2 events (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper continued over no less than four weeks). MK3475 (pembrolizumab) should be withheld for grade 2 hyperthyroidism, and permanently discontinued for grade 3 or 4 hyperthyroidism.

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.Treatment guidelines for subjects who experience an infusion reaction associated with administration of MK3475 (pembrolizumab) are described in Table 5.

Table 5. Infusion reactions treatment guidelines

NCI CTCAE v.4.0.3 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, and IV fluids); prophylactic medications indicated for ≤ 24 hours	Stop infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: Antihistamines, NSAIDS, acetaminophen, narcotics, and IV fluids. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator.	Subject may be premedicated 1.5 hours (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg orally (or equivalent dose of antihistamine) Acetaminophen 500-1000 mg orally (or

NCI CTCAE v.4.0.3 grade	Treatment	Premedication at subsequent dosing
	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/hour to 50 ml/hour). Otherwise, dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. Subjects who develop grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.	equivalent dose of antipyretic)
<u>Grades 3- 4</u> Grade 3 Prolonged (e.g., 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 and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: Antihistamines, NSAIDS, acetaminophen, narcotics, IV fluids, oxygen pressors, steroids, and epinephrine. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator. Hospitalization may be indicated. Subject is permanently discontinued from further trial treatment administration.	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

Common terminology criteria for adverse events: CTCAE; Intravenous: IV; National Cancer Institute: NCI; Non-steroidal anti-inflammatory agents: NSAIDS.

Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.3.5 Second Course *

All participants who stop study treatment with SD or better may be eligible for up to an additional 17 cycles (approximately 1 year) of pembrolizumab treatment if they progress after stopping study treatment from the initial treatment phase. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the participant meets the following conditions:

Either

- Stopped initial treatment with study treatment after attaining an investigator-determined confirmed CR based on RECIST 1.1, and
    - Was treated with at least 8 cycles of study treatment before discontinuing treatment, and
    - Received at least 2 treatments with pembrolizumab beyond the date when the initial CR was declared

OR

- Had SD, PR, or CR and stopped study treatment after completion of 35 administrations (approximately 2 years) of study treatment for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined radiographic disease progression by RECIST 1.1 after stopping initial treatment, and
    - No new anticancer treatment was administered after the last dose of study treatment, and

- The participant meets all of the safety parameters listed in the inclusion criteria and none of the safety parameters listed in the exclusion criteria, and
- The study is ongoing

An objective response or disease progression that occurs during the Second Course Phase for a participant will not be counted as an event for the primary analysis of either endpoint in this study.

5.3.6 Chemotherapy extravasation

As a general recommendation, in the event of extravasation, the following advice should be considered:

- Stop the infusion immediately.
- Do not remove the needle or cannula.
- Aspirate with the same needle as much infiltrated drug as possible from the subcutaneous site.
- Apply ice to area for 15 to 20 minutes every four to six hours for the first 72 hours.
- Paint the skin over the extravasated site with 100% DMSO (or hyaluronidase) four times daily for two weeks.

Watch the area closely during the following days in order to determine whether a surgical excision and skin graft is necessary

5.4 General concomitant medication and additional assistance guidelines

Concomitant treatment and prior medication are defined as non-investigational medicinal product (IMP). Concomitant treatment includes any prescribed medication or phytotherapy between the 28 days prior to the administration of the first treatment dose and the last safety visit during treatment period. All concomitant treatments will be recorded. After this time, information will only be collected on any anti-cancer drugs taken by the patient until end of study.

Information on concomitant medication will include start date, end date, brand or generic name, route of administration, dose, and treatment indication. The following concomitant treatments are permitted during the study:

- Erythropoiesis-stimulating agents (ESA) (such as Procrit®, Aranesp®, Epogen®) for the supportive treatment of anemia. Blood transfusions are permitted during the study.
- The prophylactic use of G-CSF is not allowed during the first treatment cycle, but can be used for cases of neutropenia arising during treatment, in accordance with the National Comprehensive Cancer Network (NCCN) guidelines.

- Bisphosphonates and denosumab for the prevention of skeletal events.
- Prophylactic or therapeutic anticoagulation therapy such as low-molecular weight heparin or warfarin at a stable dose level.
- Mineralcorticoids (e.g., fludrocortisone).
- Medications for the treatment of diarrhea, nausea, anorexia, or vomiting.
- Any medications deemed necessary to ensure patient safety and well-being may be administered at the discretion of the Investigator with the excepcion of prohibited therapies contained in **Section 5.5**.

5.5 Prohibited concomitant medications

Subjects are prohibited from receiving the following therapies during the screening and treatment phase of this trial:

- Immunotherapy not specified in the protocol.
- Chemotherapy not specified in the protocol.
- Endocrine therapy.
- Radiation therapy.

Note: Radiation therapy is allowed during screening, as long as it is completed at least two weeks prior to first dose of study treatment, and may be allowed to treat a symptomatic bone lesion or the brain after consultation with Sponsor.

- Herbal supplements.
- Live vaccines within 30 days prior to first dose of study treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella, herpes zoster, yellow fever, rabies, BCG, and typhoid (oral) vaccines. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines, and are not allowed.
- Immunosupresive agents within seven days prior to first dose of study treatment.
- Steroids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology.

Note: The use of physiologic doses of steroids (≤ 10 mg prednisone daily) is allowed and does not require Sponsor consultation.

Note: The use of systemic steroids to premedicate patients for whom computerized tomography (CT) scans with contrast are contraindicated is allowed. Magnetic resonance imaging (MRI) of

abdomen and pelvis with a non-contrast CT scan of the chest may also be performed in these patients.

Note: Local applications and inhaled steroids for management of asthma.are allowed.

Subjects who, per Investigator's assessment, require any of the aforementioned medications for clinical management should be discontinued from study treatment. There are no prohibited therapies during the post-treatment follow-up phase.

5.6 Medication errors and overdose

Medication errors in this study may arise when the drug is administered at the wrong time or when the wrong dose strength is taken. Patient medication errors should be recorded on the relevant section in the case report form (CRF). In the event of an error in the administration of the medication, the Sponsor should be informed immediately.

Medication errors must be reported irrespective of the presence of an associated AE/SAE, including:

- Medication errors involving patient exposure to the IMP.
- Any possible medication errors or use of the medication not defined in the protocol which implicate the participating patient or not.

Regardless of whether the medication error is accompanied by an AE or not, in the judgment of the Investigator, the medication error should be recorded in the dose administration record page (DAR).

5.7 Treatment compliance

Patients will receive treatment under physician supervision. Personnel will check the administration volume and total administered dose. The administered dose of each treatment will be recorded in the source data and the appropriate CRF.

6 STUDY ASSESSMENTS AND PROCEDURES

6.1 Informed consent form

Written informed consent from the patient must be signed before performing any study procedure. However, tumor assessments available and performed as part of clinical practice prior to obtaining informed consent and within 28 days prior to treatment start may be used; such evaluations do not need to be repeated for screening.

By giving their consent, patients will be informed as to the nature of the study drug and will receive pertinent information regarding the study objectives, possible benefits, and potential AEs. They will also receive information on the follow-up procedures and possible risks they will be exposed to. This document also informs patients about how biological samples will be obtained and collected and its legal implications. After receiving the document, the patient will read it (or receive information verbally before witnesses) and will sign the previously approved informed consent. The patient will receive a signed copy of the informed consent. The patient can withdraw her consent and discontinue the study, this will not affect any future medical treatment. One re-screening is allowed in patients that are screening failure in this study. Patient has to re-consent ICF before any study procedure is done.

At inclusion:

- The Sponsor will request the patient's demographic and clinical data related to screening criteria. .
- Each patient will be given a Unique Patient Number (UPN) for this study, provided by the Sponsor. All data will be recorded in the appropriate CRF using this identification number. This number will be provided to the central laboratory to ensure traceability of study samples.

Confirmation of patient's eligibility for study participation will be recorded on the CRF. The Investigator is responsible for safeguarding patient information (e.g., age, name, address, telephone number, social security number, and study identification number), ensuring access to this information by HAs if necessary. These records will remain confidential for the period of time stipulated by current legislation.

6.2 Visit schedule

All screening tests and evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria within 28 days prior to the first administration of study medication (dosing). Tumor assessments available and performed as part of clinical practice prior to obtaining informed consent and within 28 days prior to treatment start may be used; such evaluations do not need to be repeated for screening.

The Investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

Visits are organized in programmed cycles of 21 days (if there are no delays in treatment owing to the occurrence of an AE). All visits must occur within ± 2 working days (± 1 working day in day 8 of each cycle) from the scheduled date, unless otherwise noted in the schedule of assessments.

Assessments scheduled for day 1 (before treatment) of all cycles must be performed within 48 hours prior to study treatment administration and day 8 (before treatment) of all cycles must be performed within 24 hours prior to study treatment administration, unless otherwise indicated in the schedule of assessments, to confirm to the patient if treatment can be followed up. If a mandatory procedure described in the protocol falls on a bank holiday and/or weekend, this procedure should be performed on the day before or after the holiday (i.e. within a period of ± 2 working days). The summary of all study assessments are included in **Appendix 1**.

Day 8 visits can be omitted, at the Investigator's discretion,in those patients that permanently discontinue eribulin treatment and continue with pembrolizumab as single agent.

End of treatment visit (EOT) will be performed within 30 days after last study dose and End of Study Visit (including survival status and post-study anticancer therapy evaluation) will take place 12 months after last study dose in the First Course of pembrolizumab phase treatment (± 14 days). Telephone contact is acceptable.

Participants who are eligible for retreatment/crossover with pembrolizumab (as described in Section 5.3.5) may have up to two EOT visits, one after the Initial Treatment Period and one after the Second Course Treatment.

Participants who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 5.3.5 will move to the Second Course Phase when they experience disease progression. The same visits and evaluation calendar of the First Course will be followed during the Second Course Phase.

Follow-up visits

A follow up visit is required as per protocol to be performed to patients that finish the First Course Phase and are candidates to enter to the Second Course Phase of re-treatment with pembrolizumab, once disease progression is confirmed. A eCRF page will have to be completed in order to confirm the progression date. The tumor evaluation performed to confirm the progression disease before entering the Second Course Treatment will also be recorded at the eCRF.

During the period between the First and Second Course phases, it is highly recommended to perform safety follow up visits every 6 weeks and efficacy assessments every 12 weeks. However, patient's follow up during this period will be done as per local practice and at the Investigator criteria.

6.3 Demographic data and medical history

Demographic data include age, sex, and self-reported race/ethnicity. Medical history comprises clinically significant diseases, surgical interventions, history of cancer (including prior antineoplastic treatments and procedures), history of smoking, alcoholism, drug addiction, as well

as any medications (e.g., prescribed drugs, over-the-counter drugs, medicinal plants, homeopathic remedies, or food supplements) used by the patient in the 28 days prior to screening visit.

6.4 Efficacy assessments

6.4.1 Tumor and response evaluations

All measurable and evaluable lesions should be assessed and documented at the screening visit and re-assessed at each subsequent tumor evaluation. Tumor assessments will be performed at screening, every 9 weeks (± 7 days) for the first 12 months following first dose of study treatment, and every 12 weeks (± 7 days) thereafter until disease progression, treatment discontinuation, withdrawal of consent, the start of new anticancer treatment, death, or study termination by the Sponsor, whichever occurs first. The same radiographic procedures and technique must be used throughout the study for each patient.

Radiologic imaging performed during the screening period should consist of 1) CT scan of the chest, abdomen, and pelvis (MRI of the abdomen and pelvis with a non-contrast CT scan of the chest in patients for whom CT scans with contrast are contraindicated), 2) bone scan if a subject has a known history of bone metastases or has new bone pain during screening, and 3) any other imaging studies as clinically indicated by the treating physician [brain imaging during the trial should be performed in subjects with known brain metastases (every nine weeks for first year, then every 12 weeks) and those with worsening and/or new neurological symptoms].

Tumor assessments performed after the screening period should consist of 1) CT scan of the chest, abdomen, and pelvis (MRI of the abdomen and pelvis with a non-contrast CT scan of the chest in patients for whom CT scans with contrast are contraindicated), 2) bone scan if a subject develops new or worsening symptoms or if the site believes they have attained a complete response, and 3) any other imaging studies felt to be clinically indicated by the treating physician. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation.

After disease progression by RECIST v.1.1, if the site Investigator determines the subject is clinically stable and will benefit from continued treatment, the subject will then be managed by irRECIST as described in **Section 6.4.4** of the protocol.

6.4.2 Objective response assessment

CBR is defined as the proportion of the subjects in the analysis set who have a complete response, partial response, and stable disease using RECIST v.1.1 for at least 24 weeks.

ORR is defined as the proportion of the subjects in the analysis set who have a complete response or partial response based on RECIST v.1.1:

- Complete response: Complete disappearance of all targets lesions. Any pathological lymph nodes (target or non-target) must have reduction in short axis to < 10 mm.
- Partial response: At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Stable disease: Neither sufficient shrinkage to qualify for partial response, nor sufficient increase to qualify for disease progression, taking as reference the smallest sum diameters while on study.
- Disease progression: At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum diameters while on study. The development of new, previously undetected lesions, is also considered progression.

DoR is defined as the time from documentation of tumor response until disease progression or death from any cause.

6.4.3 OS and PFS definitions

OS is defined as the time from first dose of study treatment to death due to any cause. Subjects without documented death at the time of the final analysis will be censored at the date of the last follow-up.

PFS is defined as the time from first dose of study treatment to the first documented disease progression based on RECIST v.1.1 or death due to any cause, whichever occurs first.

6.4.4 irRECIST

Immunotherapeutic agents such as MK3475 (pembrolizumab) may induce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns observed with these compounds may be different to the typical time course of responses seen with classical cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST v.1.1 may, therefore, not provide a precise response assessment of immunotherapeutic agents such as MK3475 (pembrolizumab) .

irRECIST is RECIST v.1.1 adapted to account for the unique tumor response seen with immunotherapeutic agents. In this way, in subjects who have initial evidence of radiological disease progression by RECIST v.1.1, it is at the discretion of the Investigator whether to continue a subject on study treatment until repeat imaging is obtained. Subjects may receive study treatment and tumor asessment should be repeated at least four weeks later in order to confirm disease progression by irRECIST. Any subject deemed clinically unstable should be discontinued from trial treatment at first radiologic evidence of disease progression and is not required to have repeat imaging for disease progression confirmation. Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant disease progression, including worsening of laboratory values.

- No decline in ECOG performance status.
- Absence of rapid progression of disease.
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

In determining whether or not the tumor burden has increased or decreased per irRECIST, the local site Investigator should consider all target and non-target lesions as well as any incremental new lesion(s). Scenarios where disease progression is confirmed at repeat imaging if any of the following occur by irRECIST:

- Tumor burden remains ≥ 20% and at least 5 mm absolute increase compared to nadir.
- Non-target disease resulting in initial disease progression is worse (qualitative assessment).
- New lesion resulting in initial disease progression is worse (qualitative assessment).
- Additional new lesion(s) since last evaluation.
- Additional new non-target progression since last evaluation.

If repeat imaging confirms disease progression due to any of the scenarios listed above, subjects will be discontinued from study therapy. If repeat imaging does not confirm disease progression by irRECIST and the subject continues to be clinically stable, treatment may continue and follow the regular imaging schedule.

Note: If a subject with confirmed radiographic progression per irRECIST, but the subject is achieving a clinically meaningful benefit, and there is no further increase in the tumor burden at the confirmatory tumor imaging, an exception to continue treatment may be considered following consultation with the Sponsor. In this case, if treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in ***Appendix 1****.*

Note: In subjects who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging following the intervals as outlined in ***Appendix 1*** *until the start of new anti-cancer treatment, disease progression, death, or the end of the study, whichever occurs first.*

6.5 Safety and tolerability assessments

6.5.1 Laboratory assessments

Laboratory tests will be performed in accordance to local standard treatment and clinical indications. These values will include:

- Hematological test [hemoglobin, hematocrit, red blood cell count, platelet count, white blood cell (WBC) with differential count (ANC, lymphocytes, monocytes, eosinophils and basophils)], coagulation, chemistry with renal function analysis (serum creatinine), liver function [AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total and direct bilirubin], glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, and lactate dehydrogenase.
- Urinalysis (specific gravity, pH, glucose, protein, ketones, and blood).
- Thyroid function testing [thyroid-stimulating hormone (TSH), free T3, and free T4].
- HIV serology.
- HBV serology [HBsAg, anti-HBsAg].
- HCV serology (anti-HCV). In patients with a positive anti-HCV, HCV RNA detection and quantification by PCR will be additionally performed.

6.5.2 Pregnancy and assessment of fertility

MK3475 (pembrolizumab) and eribulin may have adverse effects on a fetus in utero. Only female patients of childbearing potential must undergo a pregnancy test at screening (serum pregnancy test within 14 days before Cycle 1, Day 1) to confirm eligibility in the trial and thereafter every two cycles (cycle 3 day 1, cycle 5 day 1, cycle 7 day 1, etc.) (urine pregnancy test) and at EOT visit. In case an additional pregnancy test is indicated during the trial, a serum test should be performed.

Post-menopausal status is defined as:

- Prior bilateral oophorectomy.
- Age ≥ 60 years.
- Age < 60 years and amenorrhea for 12 or more months (in the absence of chemotherapy, tamoxifen, toremifen, or ovarian suppression), and with a documented estradiol level in the postmenopausal range according to local institutional/laboratory standard.

Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use a highly effective, non-hormonal form of contraception (such as surgical sterilization, total abstinence or placement of an intrauterine device (IUD) or intrauterine system (IUS) or two effective forms of non-hormonal contraception, or are considered highly unlikely to conceive (post-menopausal; not

heterosexually active for the duration of the study) [35]. The following non-hormonal methods of contraception are acceptable:

- Condom with spermicidal foam/gel/film/cream/suppository.
- Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.

Subjects should start using birth control from screening throughout the study period up to 120 days after the last dose of treatment with MK3475 (pembrolizumab) .

In case of pregnancy, the patient must permanently stop study treatment immediately, withdraw from the trial, and the pregnancy must be reported within 24 hours to the Sponsor. The site will contact the subject at least monthly and documented the subject´s status until the pregnancy has been completed or terminated. The outcome of pregnancy will be reported to the Sponsor without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study Investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor.

6.5.3 Cardiac function monitoring

Cardiac function monitoring will consist of LVEF measurement, standard 12-lead ECG, and cardiac signs or symptoms collection. All patients must have a standard 12-lead ECG and an LVEF measurement of at least 50% by (preferably) ECHO or MUGA scan, a maximum of 28 days prior to first dose of study treatment. Additional LVEF assessments or ECGs can be performed if clinically are indicated.

6.5.4 Physical examination

A complete physical examination will include an examination of head, eyes, ears, nose, and throat, breast and locoregional lymph nodes, as well as cardiovascular, dermatological, musculoskeletal, respiratory, digestive, genitourinary, and neurological systems. A limited physical exam will consist of a symptom-directed physical examination.

Changes to abnormalities identified during the baseline period should be recorded at all subsequent physical examinations. New or worsening abnormalities should be recorded as AEs, if applicable.

6.5.5 Vital signs

These will include the measurement of height (only during screening), weight, respiratory rate, heart rate, blood pressure, and body temperature. Abnormal or significant changes in vital signs from baseline should be recorded as AEs, if appropriate.

6.5.6 ECOG performance status

Performance status will be determined using the ECOG performance status scale (see Table 6). Wherever possible, the patient's performance status should always be assessed by the same personnel throughout the study.

Table 6. ECOG performance status scale

Grade	Scale
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 house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead.

(http://www.ecog.org/general/perf_stat.html)

6.6 Translational research

The molecular study involves the collection, processing, temporary storage, and shipment of samples from consenting patients enrolled in centers selected for participation in the study. The study plan includes collection and initial processing of tumor tissues and blood samples to the central laboratory of Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), that will be used to identify dynamic biomarkers that may be predictive of response to MK3475 (pembrolizumab) and eribulin treatment.

Since the identification of new markers that correlate with disease activity and the efficacy or safety of treatment is rapidly developing, the definitive list of analyses remains to be determined, but may include determination of markers of tumor genesis pathways or mechanisms of response to immunotherapies.

6.6.1 Tumor tissue samples

Tumor samples from the metastatic tumor (biopsy) will be collected for analysis of PD-L1 status and assessment of tumor tissue biomarkers for MK3475 (pembrolizumab) and eribulin response

prediction (gene expression profiles and mutational profile through a genes' panel study will be evaluated).

Formalin-fixed paraffin-embedded (FFPE) tissue biopsies will be analyzed for PD-L1 expression by IHC at a central laboratory from a newly obtained core or excisional biopsy since last progression of a metastatic tumor lesion not previously irradiated.

PD-L1 positivity will be defined as PD-L1 expression in ≥ 1% tumor cells or in stroma for the purposes of this study.

For PD-L1 evaluation, it would be preferable to use the FFPE blocks.

Subjects for whom tumor biopsies cannot be obtained (e.g., inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor. An archival tumor block or 30 slides can be submitted to the central lab (IMIM- Institut Hospital del Mar d'Investigacions Mèdiques).

6.6.2 Blood samples

Three blood (plasma and serum) samples for translational study will be obtained at each time point; at screening, every three cyles of treatment (Cycle 4 day 1, cycle 7 day 1, Cycle 10 day 1, etc), and at the end of the study treatment in the first course in order to monitor the mutations detected in tumor samples.

- One sample (approx. 8-10 mL) will be collected in a non-EDTA, SST gel containing tube (biochemistry) for serum processing.
- Two samples (approx. 8 mL) will be collected in Cell-free DNA BCT tubes (STRECK) for plasma processing.

The processing of blood samples at the participating centers will be performed according to the protocol described into the laboratory manual (in a separate document).

6.6.3 Storage

Sample collection, storage, distribution, and destination Samples (tumor tissue and blood samples for the translational research will be sent to a central sample storage facility (MARBiobanc) located at IMIM (Hospital del Mar Medical Research Institute, Barcelona). These samples will be used for the analysis planned in the study. In addition, as scientific discoveries could happen in the future, samples collected now could be useful for future studies.

For this reason, if the patient consents, samples not used on the analysis planned in the present study will be maintained in a research line collection. In this case, patient's samples can be used for future research in breast cancer for analysis not strictly related to the objectives of the current research, sensivity to cancer or other diseases.

Samples will be maintained up to 15 years. When (or before) the 15-year period ends, patient's samples will be either destroyed or transferred to a biobank. The biobank to be used in this study

(MARBiobanc) is a public non-profit organization that gathers various biological samples collections for biomedical research purposes.

6.7 [redacted]

[redacted]

6.8 Discontinuation of patient, study, or site participation

6.8.1 Patient discontinuation

Patients have the right to withdraw from the study at any time for any reason. The Investigator also has the right to withdraw patients from the study in the event of intercurrent illness, AEs, and treatment failure after a prescribed procedure, protocol violation, administrative reasons, or for other reasons. An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of patients should be avoided.

Should a patient decide to withdraw, all efforts should be made to complete and report the observations as thoroughly as possible. The Investigator should contact the patient or a responsible relative by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the patient is withdrawing from the study. If the reason for removal of a patient from the study is an AE, the principal specific event will be recorded on the CRF.

In the case that the patient decides to prematurely discontinue study treatment, she should be asked if she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

6.8.2 Study and site discontinuation

The Sponsor reserves the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or seriousness of AEs in this or other studies indicates a potential health risk to patients.
- Patient enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- Excessively slow recruitment.
- Poor protocol adherence.
- Non-compliance with the International Conference on Harmonization (ICH) guideline for Good Clinical Practice (GCP).

7 SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

Safety assessments will consist of monitoring and recording protocol-defined AEs,)events of Clinical Interest (ECI), and SAEs; measurement of protocol-specified hematology, clinical chemistry, measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug(s).

The Sponsor or its designee is responsible for reporting relevant SAEs to competent authorities, other applicable regulatory authorities, and participating investigators, in accordance with International Conference on Harmonization (ICH) guidelines, , and/or local regulatory requirements.

The Sponsor or its designee is responsible for reporting unexpected fatal or life-threatening events associated with the use of the study drug(s) to the regulatory agencies and competent authorities within seven calendar days after being notified of the event. The Sponsor or its designee will report other relevant SAEs associated with the use of the study medication to the appropriate competent authorities (according to local guidelines), investigators, by a written safety report within 15 calendar days of notification.

7.1 AEs definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, regardless of whether it is considered related to the IMP or not.

An abnormal test finding should only be reported as an AE if meets any of the following criteria:

- Is associated with accompanying symptoms and a general diagnostic term, including the symptoms and the abnormal test finding, cannot be defined.
- Requires additional diagnostic testing or medical/surgical intervention, leads to a change in study drug(s) dosing or discontinuation from the study.
- Needs additional concomitant drug treatment.
- Is considered to be an AE by the investigator or by the Sponsor.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as the AE, and the resulting appendectomy should be recorded as treatment of the AE

The causal relationship between an AE and the IMP will be defined as follows:

- Unrelated: The temporal association between the AE and the administration of the IMP makes a causal relationship unlikely, or the subject/patient's clinical state or the study procedure/conditions provide a sufficient explanation for the AE.
- Related: The temporal association between the AE and the administration of the IMP makes a causal relationship possible, and the subject/patient's clinical state or the study procedure/conditions do not provide a sufficient explanation for the AE.

Each AE must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to the IMP.

The descriptions and grading scales found in the revised NCI CTCAE v.4.0.3 will be utilized for all toxicity reporting. A copy of the NCI CTCAE v.4.0.3 can be downloaded from the CTEP website: (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-4_QuickReference_5x7.pdf).

The intensity (severity) of an AE will be recorded as one of the following:

- Mild-easily tolerated: It does not interfere with normal daily activities. CTCAE grade 1.
- Moderate: It causes some interference with daily activities; may require intervention or treatment. CTCAE grade 2.
- Severe: Normal daily activities are substantially impaired; hospitalization and/or intervention or treatment is required. CTCAE grade 3 or 4.
- Fatal-death. CTCAE grade 5.
- Not applicable: Clinically significant and asymptomatic laboratory test abnormalities or abnormal assessments, for which no CTCAE grading guidance is applicable but which are considered as AEs.

A mild, moderate, or severe AE may or may not be serious (see definition below). These terms are used to describe the intensity of a specific AE. However, a severe AE (such as severe headache) may be of relatively minor medical significance and is not necessarily serious. For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Fever of 39°C that is not considered severe may become serious if it prolongs hospital discharge by a day. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

7.1.1 SAEs

Per definition, a SAE is defined as any AE that either:

- Results in death (i.e., the AE actually causes or leads to death).
- Is life-threatening (i.e., the AE, in the view of the investigator, places the subject/patient at immediate risk of death when it occurs).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person ability to conduct normal life functions).
- Constitutes a congenital anomaly/birth defect (in a neonate/infant born to a mother exposed to the investigational product(s).

Definition of life-threatening: An AE is life-threatening if the subject/patient was at immediate risk of death from the event as it occurred, i.e. does not include an event that might have caused death if it had occurred in a more serious form. For instance, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug induced hepatitis can be fatal.

Definition of hospitalization: AEs requiring hospitalization should be considered serious. In general, hospitalization means that the subject/patient has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment which would not have been appropriate at the study site. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered as serious.

Hospitalization for elective surgery or routine clinical procedures, which are not the result of an AE, do not need to be notified according to immediate reporting criteria. If anything untoward is reported during any procedure, this must be reported as an AE and either ‘serious’ or ‘non-serious’ attributed according to the usual criteria.

According to immediate reporting criteria, a hospitalization or prolongation of hospitalization in the absence of a precipitating clinical AE, do not need to be notified. Some examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new AE or with a worsening of the preexisting condition (i.e., for work-up of persistent pre treatment lab abnormality).
- Social admission (i.e., subject/patient has no place to sleep).
- Administrative admission (i.e., for yearly physical examination).
- Protocol-specified admission during a study (i.e., for a procedure required by the study protocol).
- Optional admission not associated with a precipitating clinical AE (i.e., for elective cosmetic surgery).
- Hospitalization for observation without a medical AE.
- Pre-planned treatments or surgical procedures should be noted in the baseline documentation and/or for the individual subject/patient.
- Admission exclusively for the administration of blood products.

Definition of clinically/medically significant event: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject/patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. Clinically/medically significant events MUST be reported as SAEs.

In this clinical trial and as defined in this protocol, SAEs and hospitalizations unequivocally and solely related to established tumour disease progression will NOT be treated as SAEs for reporting obligations.

7.1.2 Events of Clinical Interest (ECI)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor.

For the time period beginning when the consent form is signed until treatment allocationfirst dose of study treatment, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocationfirst dose of study treatment through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck, must be reported within 24 hours to the Sponsor.

Events of clinical interest for MK3475 (pembrolizumab) include:

1. An overdose of MK3475 (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.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

All SAEs/ECIs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject/patient's participation in the study, must be followed until any of the following occurs:

- The event resolves.
- The event stabilizes.
- The event returns to baseline, if a baseline value/status is available.
- The event can be attributed to agents other than the investigational product or to factors unrelated to study conduct.
- It becomes unlikely that any additional information can be obtained (subject/patient or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).

7.2 AEs reporting and other safety related issues reporting

For serious and non-serious AEs, the reporting period to the Sponsor (or its designated representative) begins from the time that the patient provides informed consent. .

Reporting period for **SAEs/ECIs that are NOT related** with the study IMPs (MK3475 (pembrolizumab) and/or eribulin) and also **all non-serious AEs** is as follows:

If patient discontinues treatment during the study, until 30 calendar days after the last administration of any of the study IMP.

All study patients will be carefully monitored for the occurrence of AEs (including SAEs and ECIs) during the above specified adverse event reporting period.

If the investigator becomes aware of a **SAE/ECI** at any time after the end of administration of study treatment and believes that it is **POSSIBLY RELATED to MK3475 (pembrolizumab)**

and/or eribulin (a serious adverse reaction to MK3475 (pembrolizumab) and/or eribulin), the investigator should notify the serious adverse reaction to the Sponsor immediately irrespective of the time elapsed since last administration of the study IMP.

For all ≥ grade 3 AEs with causal relationship to the investigational product, a follow-up by the Investigator may be required until the event or its sequelae resolve or stabilize at the acceptable level to the Investigator, and the Sponsor concurs with that assessment.

Clearly related signs, symptoms, and abnormal diagnostic procedure results should be grouped together and reported as a single diagnosis or syndrome whenever possible. Any additional events that fall outside this definition should also be reported separately.

All AEs must be recorded in the CRF.

SAE reporting and timeframe

Reporting requirements will comply with all EU safety reporting requirements as detailed in current legislation and all applicable local regulations for safety reporting.

The investigator or investigator's team will report all protocol defined SAEs and ECIs to the Sponsor (MedSIR) no later than 24 hours of any site study team staff becoming aware of the event as follows:

- The full details of the SAE and/or ECI should be collected and fully documented using the SAE form and sent to the Sponsor (MedSIR).
- Follow-up information, copies of any relevant test results, event outcome and the opinion of the investigator as to the relationship between the IMP and the SAE and ECI, accompanied by other applicable documentation when it is requested, will be sent along with the SAE form, if available on the day the event is reported or as soon as possible if it is not.
- The original SAE reporting form and the confirmation from the Sponsor must be kept with the CRF documentation at the study site(s).

All SAE forms will be sent by the investigator or investigator's team to the Sponsor (MedSIR) according to the reporting instructions provided by MedSIR at the site initiation visit and filed in the Investigator's File.

SAEs and ECIs will be followed until resolved, a stable outcome is reached, subject/patient is lost to follow-up, or dies.

As sponsor, MedSIR will be responsible for ensuring that events are reported within the mandated timeframe to the European Medicines Agency (EMA), other competent authorities, IRBs/ECs, and investigator(s), as necessary and in accordance with all applicable guidelines, approved directives and regulations. All safety reporting local regulatory requirements will be followed.

Expedited reporting to HAs, investigators, IRBs, and ECs

To determine reporting requirements for single AAE cases, MedSIR (as Sponsor) or its designee will assess the expectedness of these events using the following reference documents:

- MK3475 (pembrolizumab) Investigator Brochure (IB).
- Halaven® Summary of Product Characteristics (SmPC).

MedSIR (as Sponsor) or its designee will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

Within seven calendar days after being notified of the event, MedSIR (as Sponsor) or its designee will report unexpected fatal or life-threatening events associated with the use of the study drug to the regulatory agencies and competent authorities, to the investigators and Ethics Committees (ECs). MedSIR (as Sponsor) or its designee will report other unexpected SAEs associated with the use of the study medication to the appropriate competent authorities (according to local guidelines), investigators, and Ethics Committees (ECs) by a written safety report within 15 calendar days of notification. All safety expedited reports will be reported in accordance to all regulatory reporting obligations (including timelines) and local regulatory requirements.

Other safety-related reports

As Sponsor, MedSIR will assess constantly the benefit/risk rate of the trial, that means a continuous evaluation of the safety profile of the drugs under investigation will be done using all available information. MedSIR will provide the regulatory agencies and competent authorities and the investigators with any relevant information that may affect the benefit/risk rate of the trial. An annual DSUR safety report for study IMPs (the combination of MK3475 (pembrolizumab) and eribulin) will be prepared and distributed by MedSIR or its designee in accordance to all regulatory reporting obligations and local regulatory requirements.

In order to ensure the correct and necessary exchange of safety related information between MedSIR (as Sponsor), MSD and Eisai (as the Marketing Authorization Holders of the IMPs), a contract will be established and signed between MedSIR and these companies.

MedSIR or its designee will report any finding of noncompliance (as failure to follow any applicable regulation or institutional policies that govern human subjects' research) and/or serious noncompliance (as noncompliance that materially increases risks that result in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants) according to any reporting obligation and local regulatory requirements.

Pregnancy reporting

Irrespective of the treatment received by the subject/patient, any subject/patient's or subject/patient's partner pregnancy occurring during study treatment or during the 7 months following study drug discontinuation must be reported within 24 hours of investigator's knowledge of the event.

Pregnancies will be treated as SAEs and the investigator will complete a pregnancy form, and forward it to the sponsor according to the reporting instructions provided by MedSIR at the site initiation visit and filed in the Investigator's File.

The subject/patient will be asked to provide follow-up information on the outcome of the pregnancy, including premature termination should the case arise. Spontaneous miscarriage and congenital abnormalities will also be reported as SAEs.

The follow-up period will be deemed to have ended when the health status of the child has been determined at 12 months of the infant's life.

Additional follow up information on any MK3475 (pembrolizumab) /eribulin-exposed pregnancy and infant will be requested at specific time points (i.e., after having received the initial report, at the end of the second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant's life).

Follow-up queries may be sent, asking for further information, if required for a comprehensive assessment of the case.

8 STATISTICAL CONSIDERATIONS AND STATISTICAL ANALYSIS PLAN

8.1 Determination of sample size

A sample size of 44 evaluable patients will be accrued, with an exact single-stage phase II design. The primary endpoint for this study is the CBR. CBR will be evaluated according to the RECIST criteria v.1.1. The CBR of MK3475 (pembrolizumab) in combination with eribulin will be assessed with the exact binomial test. In designing the trial, a CBR of 30% is deemed to be unacceptable in this patient population [16]. In contrast, a CBR of 50% would be considered to be successful. At least 17 patients with clinical benefit among 39 patients will be adequate to justify the investigation of this strategy in further clinical trials. Considering a drop-out rate of 10%, a sample size of 44 patients will be needed to attain 80% power at nominal level of one-sided alpha of 0.05.

8.2 Statistical plan

8.2.1 Primary efficacy endpoint

The primary endpoint is to evaluate the CBR. The CBR is defined as the number of patients with complete response, partial response, or stable disease for at least 24 weeks divided by the number of patients in the analysis set. Tumor response will be assessed every nine weeks for the first 12 months following first dose of study treatment, and every 12 weeks thereafter. Tumor response will be defined as best response, based on local Investigator's assessment according to the RECIST criteria v.1.1.

8.2.2 Secondary efficacy endpoints

The secondary efficacy variables are PFS, OS, ORR, and DoR. These secondary efficacy endpoints have been previously defined in Sections 6.4.2 and 6.4.3.

8.2.3 Exploratory endpoints

[redacted]

8.2.4 Analysis sets

Full analysis set

All patients that accomplish selection criteria and receive at least one drug exposure.

Per-protocol set

All patients that accomplish selection criteria, receive at least one drug exposure, and receive the protocol required study drug exposure and processing. Criteria for determining the "per protocol" group assignment would be established by the Steering Committee before the statistical analysis begins.

This analysis will only occur if this set differs by ≥ 10% from the full analysis set.

Intent-to-treat set with PD-L1 positive tumors

All patients with PD-L1 positive tumors that accomplish selection criteria and receive at least one drug exposure.

Safety set

The safety set includes patients who receive at least one dose of study medication.

8.2.5 Analysis of baseline and demographic variables

The demographics and baseline characteristics will be summarized using descriptive statistics.

8.2.6 Primary efficacy analysis

We will describe number and proportion of patients with clinical benefit. We will estimate the proportion of patients with clinical benefit with the 95% Pearson-Clopper CI (95%CI).

In order to assess the robustness of the primary analysis based on the full analysis set, the primary analysis will be repeated for the per-protocol set.

8.2.7 Decision rules and adjustment of alpha for primary endpoint

- The study will be declared positive in the analysis of CBR if the one-sided p-value estimated in accordance with exact binomial test is ≤ 0.05. With this criteria, we will reach a positive finding if ≥ 17 patients achieve a clinical benefit among 39 patients.
- With this design, there is an 80% power of a positive finding if the true CBR is ≥ 50%. The one-sided type I error is 0.05.

8.2.8 Secondary and exploratory analysis

[redacted]

[redacted]

8.2.9 Biomarkers analysis

Markers will be evaluated on a univariate level regarding their potential for prediction of the clinical endpoints (CBR and ORR). Biomarker and response correlations with clinical covariates will be investigated. It will be checked whether covariates can improve the prediction and whether there is an interaction with the biomarkers. Relevant covariates could become a part of the statistical prediction model. Further multivariate techniques (i.e., Multiple Logistic Regression, Cox regression, Principal Component Analysis with Rotation, Cluster Analysis) will be considered in order to study combinations of markers. Techniques to control false discovery rate and overfitting (cross-validation) will be deliverated.

8.3 Safety analysis

Analysis of safety-related data will be considered at three levels:

- First, the degree of exposure (dose, duration, number of patients) will be assessed to determine the degree to which study safety can be assessed.
- Second, clinically relevant tests, concomitant medications, and reported AEs will be described. For AEs, severity, expectedness, causality, relationship, body system, action taken, and outcome will be reported.
- Third, SAEs, deaths, and study discontinuations for each study group will be described and assessed.

Analysis will he performed on the safety set.

8.4 Missing data management

Study variables could be missing for patients who withdrawn from the trial or for specific visits. We will report reasons for withdrawal. Patient with missing in binary outcomes will considered as non-responders. The analysis of timed endpoints are based on a Kaplan-Meier methods, therefore, not affected by patient withdrawals (as they are censored) provided that dropping out is unrelated to prognosis. The other variables will be managed with simple imputations methods (last observation carried forward). The effect that any missing data might have on results will be assessed via sensitivity analysis of study sets.

8.5 Scientific committee review

A Steering Committee has been established for this study. Initially, it is composed of the Investigators, the study medical monitor, the Scientific Global Coordinator, and two physicians expert in MK3475 (pembrolizumab) management.

The Steering Committee will meet on demand to review, discuss, and evaluate all of the gathered safety data. In case of any arising safety concern, these meetings can also be called at any time at request of a participating Investigator. At these meetings, MedSIR and the participating Investigators must reach a consensus on safety data. MedSIR will prepare minutes from these meetings and circulate them to each Investigator for comments prior to finalization.

The study site Investigators and MedSIR will review patient data at least every four months. Each study site Investigator will monitor patients data for serious toxicities on an ongoing basis.

9 ETHICAL CONSIDERATIONS

9.1 Regulatory and ethical compliance

The study will be conducted and reported in accordance with the protocol, the ICH guidelines, the ethical principles laid down in the Declaration of Helsinki, European Clinical Trials Regulation [Regulation (EU) No. 536/2014], and any applicable regulatory requirement.

9.2 EC

The conduct of the study must be authorized by a duly constituted EC. Authorization is required for study protocol, protocol amendments, informed consent forms, patient information sheets, and promotional materials. The EC must also be contacted in the event of any major protocol violation or any SAE.

Wherever necessary, the Investigator and/or Sponsor should contact the EC to ensure that accurate and timely information is being provided at every phase of the study.

The principal Investigator and/or Sponsor are responsible for providing, when required, written summaries of the study status to the EC annually or more frequently, in accordance with the requirements, policies and procedures set out by the EC. The Investigators are also responsible for immediately informing the EC of any protocol amendment.

In addition to reporting the AEs defined in the protocol to the Sponsor, the Investigators must immediately report any unexpected issues that entail a risk to patients to their respective ECs. Some ECs may request immediate reporting of all SAEs, while others may only require reporting of events if they are serious, related to the study treatment, or unexpected. The Sponsor may

provide the Investigators with written safety reports or other safety-related reports. Safety reports should be made available to the EC for review and processing in accordance with the regulatory requirements and policies and procedures established by the EC and kept on file at the study site.

9.3 Informed consent

Before any protocol-related activity, the signed informed consent should be obtained from each patient. As part of this procedure, the study site Investigator or his/her representative should explain verbally and in writing, the nature, duration, and purpose of the study, as well as the action of the drugs so that the patient is aware of the possible risks, discomfort, and adverse effects that may occur. Study patients will be informed of their right to withdraw from the study at any time and without any reason. The patient will receive all the information required by local regulations and ICH guidelines.

Consent forms must be signed and dated by the patient or their authorized legal representative before starting their participation in the study. Each patient's medical history or medical records should include an entry detailing the process of obtaining informed consent and that it was obtained in writing before the patient's participation in the study.

One copy of the signed consent form should be provided to the patient or the patient's authorized legal representative.

Signed and dated consent forms should be kept in each patient's study file. They should be made available to study monitors who may request them for review at any time.

Consent forms should be reviewed whenever changes have been made to the procedures described in the informed consent or when new information becomes available that may affect the patient's willingness to participate.

In case of review or update of consent forms, each patient's medical history should include an entry detailing the process of obtaining informed consent and that it was obtained in writing using the updated/reviewed consent form in order to continue their participation in the study. The final informed consent form approved by the EC should be provided to the Sponsor for regulatory purposes.

9.4 Data protection

The Sponsor will ensure confidentiality of the patient's medical information, in accordance with the applicable laws and regulations.

The study Sponsor, as data controller according to the European Directive on the protection of individuals with regard to the processing of personal data and on the free movement of such data confirms herewith compliance with Directive 95/46/EC, Organic Law 15/1999, and Royal Decree 1720/2007 in all stages of data management.

The data generated by this study will be made available to the representatives of national and local HAs, Sponsor monitors, Sponsor representatives, and partners, as well as the EC of each study site for review, as needed.

10 ORIGINAL DOCUMENTS, STUDY MONITORING AND QUALITY ASSURANCE

10.1 Source data record

Source data refers to all the information found in source files and certified copies of source files containing clinical findings, observations, or other activities that are part of the clinical study and which are necessary for study reconstruction and assessment. Source data is contained in source documents (comprising source documents and certified copies).

Source documents are original documents, data, and records (e.g., hospital records, medical histories and office records, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patients files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study).

The Sponsor’s quality assurance group may assist in determining whether electronic records generated using computerized medical record systems used at investigational sites can be considered source documentation for the purposes of this protocol.

If the site computerized medical record system has not been adequately validated for the purposes of clinical research (instead of general clinical practice), all paper source documentation should be kept on file to ensure that important protocol data entered on the CRFs can be verified.

At a minimum, source documentation must be available to substantiate patient identification, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; adequate reporting and follow-up of AEs; administration of concomitant medication; study drug receipt/dispensing/return records; study drug administration information; and date of completion and reason.

Data recorded on the CRF will be verified by checking the CRF entries against source documents (i.e., all original documents, laboratory reports, medical records) in order to ensure data

completeness and accuracy as required by study protocol. The Investigator and/or site staff must make CRFs and source documents of patients enrolled in this study available for inspection by Sponsor or its representative at the time of each monitoring visit.

The source documents must also be available for inspection, verification, and copying, as required by regulations, officials of the regulatory HAs (e.g., FDA, EMA, and others), and/or ECs. The study Investigator and site staff must comply with applicable privacy, data protection, and medical confidentiality laws for use and disclosure of information related to the study and enrolled patients.

The patient must also allow access to the patient's medical record. Each patient should be informed of this requirement prior to study start.

10.2 Study monitoring and source data verification

Sponsor or its representative will monitor study progress as frequently as necessary to ensure:

- Protection of study patients' rights and well-being.
- Study data are accurate, complete, and verifiable from the source documents.
- Conduct of the trial is in compliance with the approved protocol/amendment, GCP guidelines, and applicable regulatory requirements.

Study staff contact details are included in a document located on the Investigator site file

Data recorded on the CRF will be verified by checking the CRF entries against source documents (i.e., all original documents, laboratory reports, medical records, patients' diaries) in order to ensure data completeness and accuracy as required by study protocol. The Investigator and/or site staff must make CRFs and source documents of patients enrolled in this study available for inspection by the Sponsor or its representative at the time of each monitoring visit.

10.3 Record retention

Investigators should retain all study records in a secure place with restricted access as per applicable regulatory requirements. The Investigator must consult a Sponsor representative before disposal of any study records and must notify the Sponsor (of any change in the location, disposition, or custody of the study files.

Essential documents must be retained by the Investigator for at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. "Essential

documents" are defined as those documents which individually and collectively allow for the assessment of the conduct of the study and the quality of the resulting data. These documents must be retained for a longer period if required by the applicable regulatory requirements or as a result of an agreement with the Sponsor. The Committee for Medicinal Products for Human Use (CHMP) requires retention for the maximum period of time permitted by the institution, but not less than 15 years (ICH E6, 4.9.5). It is the responsibility of the Sponsor to inform the Investigator or site when these documents no longer need to be retained (ICH E6, 4.9.5).

The study site Investigator should not dispose of records belonging to this study without either (1) obtaining written approval from the Sponsor or (2) offering the Sponsor an opportunity to collect these records. The study site Investigator will be responsible for retaining suitable and accurate electronic or paper copies of source documentation containing observations and data collected from this study. Such documentation is subject to inspection by the Sponsor and the FDA and/or EMA (or respective individual EU country regulatory authorities).

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

10.4 Data quality assurance

During the study or upon its completion, the quality assurance auditor(s) designated by Sponsor or the competent authorities may wish to perform an audit of the sites. Investigators are expected to cooperate in these audits, offering their assistance, and providing auditors with the documentation they require (including source documents).

The Sponsor's representatives are responsible for contacting and visiting the Investigator in order to inspect the facilities and, upon request, to inspect clinical trial records (e.g., CRF and other pertinent data), provided that patient confidentiality is respected.

The Investigator agrees to cooperate with the monitor to ensure that all issues detected during these monitoring visits are resolved, including delays in completing CRFs.

In accordance with the ICH E6 R2 Guideline for GCP and the Sponsor's audit plans, the Sponsor's Department of Quality Assurance or its representatives may perform an audit of this study. An inspection of site facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will be carried out in order to assess the conduct of the study and compliance with the protocol, the ICH guidelines for GCP (ICH E6 R2), and relevant regulatory requirements.

11 DATA MANAGEMENT

11.1 Data entry and processing

In this study, the Investigator and/or site staff will regularly enter all data into the CRFs.The Investigator must also review all data included in the CRF to ensure its accuracy.

Reconciliation of the data will be performed by the designated CRO. At the end of the study, any protocol non-compliances will be identified and recorded as part of the clinical database. Once all of these tasks are complete and the accuracy and completeness of the database has been verified, it will be locked and the data will become available for data statistical analysis.

11.2 Data clarification

As part of the conduct of the trial, Sponsor may have questions about the data entered by the site, referred to as queries. Queries can be generated by the monitors, data managers and the Sponsor

11.3 Data coding procedures

Coding of AEs, medical history, and prior and concomitant medications will be performed using standard dictionaries described in the Data Management Plan.

12 STUDY MANAGEMENT

12.1 Study discontinuation

MedSIR reserves the right to discontinue the study at any time for safety or administrative reasons. If the study is discontinued and/or the site is closed for any reason, all of the investigational study drugs can be destroyed locally at the site according local law regarding radioactive products) . All of the actions needed to assess or maintain study patient safety will continue as required, despite study discontinuation.

12.2 Protocol amendments

Any change or addition to the protocol requires a written protocol amendment or administrative letter that must be approved by Sponsor, the Scientific Global Coordinator, the study site Investigator, and the EC/HAs when applicable. This requirement should not, under any circumstances, prevent any immediate action taken by the study site Investigator or Sponsor to ensure the safety of all the patients enrolled in the study. If, in the opinion of the study site Investigator, an immediate protocol amendment is required and it is applied for safety reasons,

this process should be reported to Sponsor as soon as possible (within 24 hours) and the EC will be informed, as needed.

12.3 Publication policy and protection of trade secrets

All of the data generated by this study should be considered highly confidential and should not be disclosed to persons who are not directly related to the study without prior written permission from the Scientific Global Coordinator and Sponsor. Nevertheless, full access is granted to authorized officials of HAs, the Scientific Global Coordinator or study site Investigator, and Sponsor staff (or their representatives) for record inspection and copying. All of the investigational products, patient body fluids and/or other collected materials will only be used in accordance with this protocol, unless otherwise agreed in writing between the global scientific coordinator or study site Investigator and Sponsor.

The Sponsor will ensure that, as far as possible, the study results are published in the form of scientific/clinical articles in prestigious scientific peer-reviewed journals. These documents will be prepared with the full participation of the principal Investigators and in accordance with applicable guidelines for Good Publication Practice and with international recommendations, such as those from the International Committee of Medical Journal Editors (ICMJE) and all elements of the Consort Statement (2010).

The Sponsor will be notified of any attempt to publish data obtained during the study and permission to do so must first be obtained from the Sponsor before publication.

12.4 Insurance

The Sponsor contracts an insurance policy to cover the responsibilities of the Investigator and other parties participating in the study, according to the applicable Spanish legislation.

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35. Recommendations related to contraception and pregnancy testing in clinical trials (CTFG)

http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf

14 APPENDIX

Appendix 1: Schedule of study assessments and procedures

Assessment Window (Days)	Screening	All cycles (First and Second Course Phase)		End Of Treatment visit (EOT)[13]	Follow up visit to confirm patient progression to Sponsor[14]	End Of Study (EOS) 12 months after last study dose in the first course of pembrolizumab phase treatment, or after the last study dose in the second course in all patients.
	Days –28 to –1	1 (± 2 days)	8[12] (± 1 day)	≤ 30 days after last dose	At patient's disease progression after First Treatment Course	
Signed informed consent form(s)	x					
Review of eligibility criteria	x					
Demographic data and medical history[1]	x					
HIV, HBV, HCV serology (± HCV RNA	x					
Concomitant medications	x	x	x	x	x	
Complete physical examination	x	x		x		

Limited physical examination (symptom-directed)			x			
Assessment of symptoms	x	x	x	x		
ECOG performance status	x	x		x		
Vital signs[2]	x	x	x	x		
Weight	x	x		x		
Height	x					
Newly obtained and/or archival tissue for biomarker analysis	x					
Tumor assessment[3]	x		See footnote ([4])			
12-lead electrocardiogram	x		Performed as clinically indicated			
FEVI assesstment (ECHO or MUGA)	x		Performed as clinically indicated			
Hematology[5]	x	x([6])	x	x		
Serum chemistry[5]	x	x([6])	x	x		
TSH, free T3, free T4	x	Every two cycles[8]				
Coagulation panel (aPTT, INR)	x			x		
Urinalysis[7]	x	Every two cycles[8]				
Pregnancy test (women of childbearing potential only)[9]	x([9])	Every two cycles[8]		x		
Blood samples for translational research[10]	x	Every three cycles		x		
[redacted]						
[redacted]						

Adverse events	x	x	x	x	x	
MK3475 (pembrolizumab) infusion		x				
Eribulin administration		x	x		X (if applicable)	
Suvival and anti-cancer therapy follow-up					x	x

Anti-therapeutic antibody: ATA; Eastern Cooperative Oncology Group: ECOG; Echocardiography: ECHO; Hepatitis B virus: HBV; Hepatitis C virus: HCV; Left ventricular ejection fraction: LVEF; Multiple-gated acquisition: MUGA; Pharmacokinetic: PK; Pharmacodynamic: PD; Thyroid-stimulating hormone: TSH.

1. Demographic data include age, sex, and self-reported race/ethnicity. Medical history comprises clinically significant diseases, surgical interventions, history of cancer (including prior antineoplastic treatments and procedures), history of smoking, alcoholism, drug addiction, as well as any medications (e.g., prescribed drugs, over-the-counter drugs, medicinal plants, homeopathic remedies, or food supplements) used by the patient in the 28 days prior to screening visit.
2. Vital signs include: respiratory rate, heart rate, blood pressure, and body temperature
3. All measurable and evaluable lesions should be assessed and documented at the screening visit. Radiologic imaging performed during the screening period should consist of 1) CT of the chest, abdomen, and pelvis or MRI of the abdomen and pelvis with a non-contrast CT scan of the chest in patients for whom CT scans with contrast are contraindicated, 2) bone scan if a subject has a known history of bone metastases or has new bone pain during screening, and 3) any other imaging studies as clinically indicated by the treating physician [brain imaging during the trial should be performed in subjects with known brain metastases (every nine weeks for first year, then every 12 weeks) and those with worsening and/or new neurological symptoms]. Tumor assessments will be performed at baseline, every 9 weeks (± 7 days) for the first 12 months following first dose of study treatment, and every 12 weeks (± 7 days) thereafter, with additional scans as clinically indicated. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation. The same radiographic procedures and technique must be used throughout the study for each patient (bone scan will be performed only if a subject develops new or worsening symptoms or if the site believes they have attained a complete

response).

4. In subjects who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging following the intervals as outlined in footnote 3 until the start of new anti-cancer treatment, disease progression, death, or the end of the study, whichever occurs first. However, tumor evaluation will be done by the Investigator criteria and local practice according to the technical sheet of pembrolizumab during the suspension of the First Course Phase and during the Second Course Phase.
5. Blood test will be performed as per local standard of care and clinical indication before treatment administration. These values should be included: hemoglobin, hematocrit, red blood cell count, platelet count, and WBC count with differential count (ANC, lymphocytes, monocytes, eosinophils, and basophils), coagulation, chemistry with renal function analysis (serum creatinine), liver function (AST, ALT, ALP, GGT, total and direct bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, and lactate dehydrogenase.
6. Not repeat in Cycle 1, Day 1 if done in day -3 to day 1.
7. Urinalysis include: Specific gravity, pH, glucose, protein, ketones, and blood
8. Every 2 cycles: Cycle 3 day 1, cycle 5 day 1, cycle 7 day 1, etc, before study treatment administration.
9. Serum pregnancy test within 14 days before Cycle 1, Day 1. Urine pregnancy test every two cycles thereafter.
10. Samples for translational study will be obtained at each time point; at screening, every three cyles of treatment (Cycle 4 day 1, cycle 7 day 1, Cycle 10 day 1, etc), and at the end of the study treatment ony in the first course.

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12. Day 8 visits can be omitted, at the Investigator's discretion, in those patients that permanently discontinue eribulin treatment and continue with pembrolizumab as single agent.

13. End of treatment visit (EOT) will be performed within 30 days after last study dose. Participants who are eligible for retreatment/crossover with pembrolizumab (as described in Section 5.3.5) may have up to two EOT visits, one after the Initial Treatment Period and one after the Second Course Treatment.
14. A follow up visit is required as per protocol to be performed to patients that finish the First Course Phase and are candidates to enter to the Second Course Phase of re-treatment with pembrolizumab, once disease progression is confirmed. A eCRF page will have to be completed in order to confirm the progression date.

    During the period between the First and Second Course phases, it is highly recommended to perform safety follow up visits every 6 weeks and efficacy assessments every 12 weeks. However, patient's follow up during this period will be done as per local practice and at the Investigator criteria.

Appendix 2: Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1

Criteria for evaluating response in solid tumors

RECIST v.1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST v.1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

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

Appendix 3: Imaging and treatment after first radiologic evidence of disease progression. Table r e presents irRECIST

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
First radiologic evidence of disease progression by RECIST v.1.1	Repeat imaging at ≥ 4 weeks at site to confirm disease progression	May continue study treatment at the local site Investigator's discretion while awaiting confirmatory tumor imaging by site by irRECIST	Repeat imaging at ≥ 4 weeks to confirm disease progression per physician discretion only	Discontinue treatment
Repeat tumor imaging confirms disease progression by irRECIST	No additional imaging required	Discontinue treatment (exception is possible upon consultation with Sponsor)	No additional imaging required	Not applied
Repeat tumor imaging shows stable disease or partial or complete response by irRECIST	Continue regularly scheduled assessments	Continue study treatment at the local site Investigator´s discretion	Continue regularly scheduled assessments	May restart study treatment if condition has improved and/or clinically stable per local site Investigator´s discretion

Immune-Related Response Evaluation Criteria In Solid Tumors: irRECIST; Response criteria in solid tumors: RECIST.

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