



IBCSG

**INTERNATIONAL BREAST
CANCER STUDY GROUP
IBCSG 45-13/BIG 4-13**

PANACEA



**A phase Ib/II trial evaluating the efficacy of MK-3475 and
trastuzumab in patients with trastuzumab-resistant, HER2-
positive metastatic breast cancers**

**PANACEA – anti-PD-1 monoclonal antibody in Advanced,
trastuzumab-resistant, HER2-positive breast cancer**

EudraCT number: 2013-004770-10

NCT Number: NCT02129556

Merck number: MK-3475 protocol 14

**Sponsor: International Breast Cancer Study Group
(IBCSG)**

**IBCSG Coordinating Center, Effingerstrasse 40, CH- 3008 Bern
Phone: +41 31 389 93 91 Fax: +41 31 389 93 92**

Amendment 1

Protocol Version 2.1



Trial Specific Contacts

Trial Chair	Assoc. Prof. Sherene Loi Department of Medical Oncology Peter MacCallum Cancer Centre St Andrew's Place East Melbourne, Victoria 3002 Australia	Email: sherene.loi@petermac.org Tel: +61 (0) 396 563 642 Fax: +61 (0) 396 561 411
Trial Co-Chair	Prof. Fabrice André Department of Medical Oncology Institut Gustave Roussy 114 rue E Vaillant 94800 Villejuif France	Email: fabrice.andre@igr.fr Tel: +33142114371 Fax: +33142115274
Translational Research Committee Co-chairs:	Prof. Mark J Smyth, Immunology in Cancer Laboratory, Queensland Institute of Medical Research, Hersten, QLD, Australia Giuseppe Curigliano, European Institute of Oncology, Milan, Italy	Email: mark.smyth@qimr.edu.au Tel: +61 7 3845 3957 Fax: +61 7 3362 0111 Email: giuseppe.curigliano@ieo.it Tel: +39 (02) 57489788 Fax: +39 (02) 94379224
Trial Statistician	Anita Giobbie-Hurder, MS IBCSG Statistical Center	Email: agiohur@jimmy.harvard.edu Tel: +1 617-632-2193 Fax: +1 617-632-5444
Lead Trial Data Manager Trial Data Manager Lead Trial Coordinator Trial Coordinator	Susan Fischer Monica Greco Holly Shaw Colleen King IBCSG Data Management Center	Email: ibcsg45_PANACEA@fstrf.org Tel: +1 716-834-0900 (general) Fax: +1 716-833-3990
IBCSG Central Pathology Office	Rosita Kammler Stefania Andrighetto/Elvira Benini	Email: rosita.kammler@ibcsg.org Email: pathology.ibcsg@ieo.it Tel: +39 0257 489 928
Central Laboratory	Quintiles Alba Campus, Rosebank Livingston, West Lothian Scotland, EH54 7EG, UK	



IBCSG Contacts

Coordinating Center	Anita Hiltbrunner Dr. Barbara Ruepp Dr. Manuela Rabaglio Dr. Rudolf Maibach Linda Fritzsche / Sabrina Ribeli- Hofmann Rosita Kammler IBCSG Coordinating Center Effingerstrasse 40 CH-3008 Bern, Switzerland	Email: anita.hiltbrunner@ibcsg.org Email: barbara.ruepp@ibcsg.org Email: manuela.rabaglio@ibcsg.org Email: rudolf.maibach@ibcsg.org Email: drugsupply@ibcsg.org Email: rosita.kammler@ibcsg.org Tel: +41 31 389 9391 Fax: +41 31 389 9392
Data Management Center	Lynette Blacher Karolyn Scott FSTRF, 4033 Maple Rd. Amherst, NY 14226 USA	Email: blacher.lynette@fstrf.org Email: scott.karolyn@fstrf.org Tel: +1 716 834-0900 Fax: +1 716 833-3990
Data Quality Control Office	IBCSG Data Management Center	Email: ib.dqc@fstrf.org
Center Training Office	IBCSG Data Management Center	E-mail: ibcsg.training@fstrf.org
Statistical Center	Prof. Richard D. Gelber Dr. Meredith M. Regan Anita Giobbie-Hurder Dept. of Biostatistics and Computational Biology, CLSB 11007 Dana-Farber Cancer Institute 450 Brookline Ave. Boston, MA 02215 USA	Email: gelber@jimmy.harvard.edu Email: mregan@jimmy.harvard.edu Email: agiohur@jimmy.harvard.edu Tel: +1 617-632-3012 Fax: +1 617-632-5444
Central Pathology Office	Prof. Giuseppe Viale University of Milan & European Institute of Oncology Milan, Italy	E-mail: giuseppe.viale@ieo.it Tel: +39 02 5748 9419 Fax: +39 02 5748 9417
Scientific Committee Chair	Dr. Marco Colleoni European Institute of Oncology Milan, Italy	Email: marco.colleoni@ieo.it Tel: +39 02 574 89 439 Fax: +39 02 574 89 581

In collaboration with Merck Sharp & Dohme Corp.



Protocol Signature Page

IBCSG 45-13

PANACEA

Approved by:

Group Statistician, International Breast Cancer Study Group
Dr. M.M. Regan

Signature on file

14 Apr 2014

Date

Approved by:

Director, International Breast Cancer Study Group
Anita Hiltbrunner

Signature on file

14 Apr 2014

Date



Protocol Signature Page Amendment 1

IBCSG 45-13

PANACEA

Approved by:

Group Statistician, International Breast Cancer Study Group
Dr. M.M. Regan

Date

Approved by:

Director, International Breast Cancer Study Group
Anita Hiltbrunner

Date



Principal Investigator Protocol Signature Page

Amendment 1

IBCSG 45-13
PANACEA

I have read the protocol and agree that it contains all necessary details for conducting this trial. I will conduct the trial as outlined in the following protocol and in compliance with GCP, and will apply due diligence to avoid protocol deviations. I will provide copies of the protocol and all drug information relating to pre-clinical and prior clinical experience furnished to me by IBCSG, **to all physicians responsible to me who participate in this trial. I will discuss this material with them to assure that they are fully informed** regarding the drugs and the conduct of the trial. I agree to keep records on all patient information (Case Report Forms and patient's Informed Consent statement), drug-shipment and return forms, and all other information collected during the trial for a minimum period of 15 years.

Name of Principal Investigator: _____

Signature

Date



TABLE OF CONTENTS

Section	Page
Trial Specific Contacts.....	2
IBCSG Contacts.....	3
1. Protocol Summary and Schema	10
2. Trial schedule	21
3. List of abbreviations	23
4. Background and scientific rationale	25
4.1. HER2-positive metastatic breast cancers	25
4.2. Rationale for combining immune checkpoint modulators and trastuzumab	25
4.3. Anti-PD-1 and PD-L1 antibodies	27
4.4. Trial hypothesis	31
4.5. Overall risk-benefit assessment	32
4.6. Rationale for the trial design	32
5. Trial objectives and endpoints.....	33
5.1. Primary endpoint	34
5.2. Secondary endpoints.....	34
5.3. Correlative objectives	34
5.4. Exploratory objectives	34
6. Trial design, duration and termination	34
6.1. Trial design	35
6.2. Trial schema	36
6.3. Clinical evaluations	36
6.4. Biological evaluations	37
6.5. Sample size and trial duration	37
7. Patient selection.....	38
7.1. Eligibility criteria for screening.....	38
7.2. Exclusion criteria for screening.....	40
7.3. Inclusion criteria for enrollment.....	41
7.4. Exclusion criteria for enrollment.....	42
8. Patient registration for screening and enrollment.....	43
8.1. Registration for screening.....	43
8.2. Prior to enrollment.....	44
8.3. Registration for Enrollment.....	44
8.4. Registration Help Desk.....	44
9. Trial drugs formulation and handling.....	45
9.1. MK-3475	45
9.2. Trastuzumab	46
10. Treatment.....	46
10.1. Trial treatments.....	46
10.2. Phase Ib: dose finding and DLTs	46
10.3. Phase II dose of MK-3475.....	48
10.4. Treatment Administration.....	48
10.5. Dose modifications and delays for trastuzumab.....	49
10.6. Dose modifications and delays for MK-3475.....	52
10.7. Concomitant Medications/Vaccinations (allowed & prohibited).....	60
10.8. Diet/Activity/Other Considerations	61
10.9. Stop of treatment	62
11. Safety.....	63
11.1. Adverse effects of MK-3475	63



11.2.	Immune-related adverse effects of MK-3475	64
11.3.	Drug interactions with MK-3475	64
11.4.	Adverse effects of trastuzumab	64
11.5.	Safety Assessment in stage 1 of phase II	64
12.	Adverse event and serious adverse event reporting	65
12.1.	Adverse event reporting	65
12.2.	Serious adverse event (SAE) reporting	67
12.3.	Definition of Events of Clinical Interest	68
12.4.	Reporting SAEs and ECIs	68
12.5.	Pregnancy	69
12.6.	Reference Safety Information	70
13.	Disease assessment, response and progression (RECIST 1.1)	70
13.1.	Introduction	70
13.2.	Methods of assessment	70
13.3.	Measurability of tumor at baseline	71
13.4.	Selection of target lesions	72
13.5.	Selection of non-target lesions	72
13.6.	Evaluation of target lesions (measurable disease)	73
13.7.	Evaluation of non-target lesions	73
13.8.	Determination of new lesions	74
13.9.	Additional considerations	74
13.10.	Confirmation	74
13.11.	Determination of time point response	74
13.12.	Determination of best overall response	75
13.13.	Storage of imaging	75
14.	Clinical and laboratory evaluations	75
14.1.	Screening	75
14.2.	Central confirmation of HER2+ and PD-L1 status	76
14.3.	Biological samples before, during and after treatment	77
14.4.	Day 1 of every treatment cycle	77
14.5.	Every second treatment cycle	77
14.6.	Tumor assessments	77
14.7.	Cardiac evaluation	77
14.8.	After discontinuation of trial treatment	78
14.9.	Follow-up prior to documented disease progression	79
14.10.	At progression	80
15.	Biological evaluations	80
15.1.	Introduction and overview	80
15.2.	Submitting pathology material to IBCSG	81
15.3.	Central testing of HER2 and PD-L1	82
15.4.	Banking biological material	82
15.5.	Translational research	83
16.	Data submission	85
16.1.	Case report forms schedule	86
16.2.	Signing and submitting forms	87
16.3.	Data management	87
16.4.	Investigator Site File	88
16.5.	Authorization Log	89
16.6.	Patient Identification Log	89
17.	Statistical considerations	89



17.1.	Primary and Secondary Endpoints	89
17.2.	Definitions of Trial Populations	90
17.3.	Design and Sample Size Determination	90
17.4.	Safety Monitoring.....	92
17.5.	Screening and Enrollment	94
17.6.	Analysis of Demographics/Baseline Characteristics	94
17.7.	Efficacy Analysis (Phase II)	94
17.8.	Correlative Objectives	95
17.9.	Exploratory Objectives	97
18.	Ethical aspects, regulatory approval, and patient informed consent	97
18.1.	Ethical Review Board/Ethics Committee	98
18.2.	Regulatory approval procedures	98
18.3.	Protection of human patients	98
18.4.	Informed Consent	98
18.5.	Premature withdrawal.....	99
19.	Governance and Administrative Considerations	100
19.1.	Insurance.....	100
19.2.	Steering Committee	100
19.3.	Data and Safety Monitoring Committee (DSMC).....	100
19.4.	Endpoint Committee.....	100
19.5.	Publication of trial results.....	100
19.6.	Premature discontinuation of the trial	101
19.7.	Quality Assurance.....	101
19.8.	Protocol adherence	101
19.9.	Data protection	102
19.10.	Record Retention	102
20.	Confidentiality.....	102
21.	References	102

Appendices

1. Requirements for Informed Consent

Index of Tables

Table 1	Dose escalation rules	47
Table 2	Body weight based dose levels	48
Table 3	Actions to be taken in case of trastuzumab related adverse events	49
Table 4	NYHA functional classification.....	51
Table 5	Treatment delay and supportive care guidelines for drug-related adverse events ...	53
Table 6	Infusion reaction treatment guidelines.....	59
Table 7	Measurable disease - overall response.....	74
Table 8	Tumor tissue samples.....	80
Table 9	Blood samples for all patients.....	81
Table 10	Operating characteristics of early cardiac monitoring.....	93
Table 11	Operating characteristics of early safety monitoring.....	93



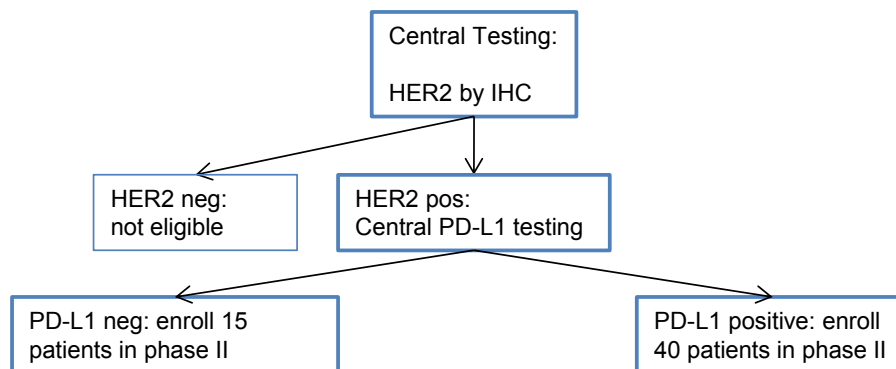
1. Protocol Summary and Schema

TITLE	A phase Ib/II trial evaluating the efficacy of MK-3475 and trastuzumab in patients with trastuzumab-resistant, HER2-positive metastatic breast cancers (PANACEA)
SPONSOR	International Breast Cancer Study Group (IBCSG)
PHARMA PARTNER	Merck Sharp & Dohme Corp.
CLINICAL PHASE	Phase Ib/II <i>Note: The phase Ib part of the trial was completed in August 2015.</i>
POPULATION	Patients with HER2-positive, PD-L1 expressing, unresectable loco-regional or metastatic breast carcinoma which has progressed on prior trastuzumab-based therapy. <i>A parallel cohort of 15 patients with HER2-positive, PD-L1 negative disease will be enrolled in the phase II study.</i>
TREATMENT	Treatment: <i>In the phase Ib trial, three MK-3475 body weight based dose levels: 2 mg/kg, 10 mg/kg, or a fall-back dose of 1 mg/kg together with trastuzumab 6 mg/kg i.v. every 3 weeks. MK-3475 at 200 mg, administered by i.v. infusion, will be the dose for the phase II together with trastuzumab 6 mg/kg i.v. every 3 weeks until progression, lack of tolerability, or 24 months of treatment.</i> A loading dose of 8 mg/kg i.v. trastuzumab in cycle 1 is needed if trastuzumab has been stopped more than 3 months before.



TRIAL SCHEMA

Screening: unresectable locoregional or metastatic breast cancer overexpressing HER2
 → Submit **an FFPE block from** core biopsy for central testing



Phase Ib: dose finding for MK-3475 in 3+3 design — **Phase II** 200mg

Treatment in 3 week cycles: 1 2 3 4 5 etc..... PD

T : trastuzumab 6mg/kg

M : MK-3475 200mg

T

M

T

M

T

M

T

M

T

M

Tissue Samples:

↑
 at enrollment:
 FFPE block
 Fresh frozen block *
 * if feasible

↑
 re-biopsy at PD *:
 FFPE Block
 Fresh frozen block
 * if feasible

Blood samples:

↑ whole blood
 ↑ plasma prior to cycles 1, 3, 5 and then every 3 cycles,
 and 30 days after end of tx

RATIONALE

There is a clear need to develop new therapeutic agents for patients presenting with HER2-overexpressing or HER2-positive metastatic breast cancer.

A significant amount of preclinical and correlative clinical data suggests that HER2-positive breast cancer could be amenable to immunotherapeutic approaches. Recently, new data have reported that tumor regressions in vivo from the anti-HER2 monoclonal antibody (mAb) therapy, trastuzumab, require an effective adaptive host anti-tumor immune response. In immunocompetent mice, combination of the anti-HER2 mAb and anti-PD-1 therapy was shown to be synergistic and more effective than either monotherapy in mammary tumors that overexpress HER2. The presence of HER2-overexpression in breast cancers is associated with higher levels of proliferation, high histologic grade and higher levels of tumor infiltrating lymphocytes (TILs) compared with HER2-negative tumors. We therefore hypothesize that for HER2-positive tumors, avoidance of destruction by the host immune system must be an important mechanism contributing to tumor growth



	<p>and progression.</p> <p>The term “immune evasion” refers to the ability of the tumor to suppress and change host anti-tumor immune reactions. The programmed cell death 1 (PD-1) pathway represents a major immune control switch, which may be engaged by tumor cells to overcome active T-cell immune surveillance. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in various tumors. High expression of PD-L1 on tumor cells (and to a lesser extent of PD-L2) has been found to correlate with poor prognosis and survival in various other solid tumor types. Furthermore, PD-1 has been suggested to regulate tumor-specific T-cell expansion in patients with malignant melanoma. These observations suggest that the PD-1/PD-L1 pathway plays a critical role in the tumor immune evasion and could be considered an attractive target for therapeutic intervention in several solid organ types.</p> <p>MK-3475 (previously known as SCH 900475) is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. MK-3475 contains the S228P stabilizing mutation and has no antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) activity. MK-3475 strongly enhances T lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. In T-cell activation assays using human donor blood cells, the EC50 was in the range of 0.1 to 0.3 nM. MK-3475 also modulates the level of interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and other cytokines. The antibody potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T-cells.</p> <p>We therefore propose to evaluate if the addition of an immunotherapy can reverse trastuzumab resistance and improve clinical outcomes in HER2-positive disease. In this study, we will determine if a monoclonal antibody targeted against PD-1, a T-cell negative regulator, can reverse trastuzumab resistance in patients previously progressing on trastuzumab. The combination will be evaluated in the metastatic setting to demonstrate safety of the combination and the single arm study approach will be used as it is standard-of-care in the HER2-positive metastatic population to continue an anti-HER2 therapy back-bone (in this case trastuzumab) even after progression. This will be the first trial that will evaluate the efficacy of an immunotherapeutic approach in breast cancer.</p>
--	--



ELIGIBILITY	<p><u>Inclusion criteria for screening:</u></p> <ul style="list-style-type: none">• Female gender• Age ≥ 18 years• Histologically confirmed breast adenocarcinoma that is unresectable loco-regional, or metastatic.• Locally confirmed HER2-positivity (immunohistochemistry score 3+) or ERBB2-amplification (Ratio ERBB2/centromeres ≥ 2.0 or mean gene copy number ≥ 6) of primary tumor or of biopsy from metastatic or unresectable loco-regional lesion.• Must have trastuzumab resistant disease, defined by<ul style="list-style-type: none">- demonstrated progression of disease while on treatment with trastuzumab (monotherapy or combination-based therapy) or trastuzumab-emtansine (T-DM1) for metastatic or unresectable loco-regional disease.- recurrence while on adjuvant trastuzumab or within 12 months of completing adjuvant trastuzumab.- Any number of prior lines of anti-HER2 therapy acceptable. Patients for whom the treatment with the current first-line combination of trastuzumab, pertuzumab and taxanes is not an option (e.g., due to refusal or contraindication) can be considered for enrollment into this trial.• Progression/recurrence must have been demonstrated by radiological or clinical assessment.• If a patient has received a subsequent anti-HER2 therapy, she must also have progressed on the subsequent therapy. Progression must have been demonstrated by radiological or clinical assessment.• Presence of at least one measurable lesion as defined by RECIST 1.1.• LVEF $\geq 50\%$• Patient agrees to submit an FFPE tumor biopsy for central confirmation of HER2-positivity and central assessment of PD-L1 status. This can be from archival tissue sample from unresectable loco-regional or metastatic disease obtained ≤ 1 year prior to enrollment or new tissue material from a recently obtained surgical or diagnostic biopsy. Tissue obtained for the biopsy must not have been previously irradiated. <u>Note:</u> Central Pathology Review on a tumor biopsy is mandatory for this trial, and patients will be evaluated centrally for eligibility.• Written Informed Consent (IC) for screening procedures and trial participation must be signed and dated by the patient and the
--------------------	---



	<p>Investigator prior to screening.</p> <ul style="list-style-type: none">• Written consent to biological material submission, indicating the patient has been informed of and agrees to tissue and blood material use, transfer and handling, must be signed and dated by the patient and the Investigator prior to any procedures specific for this trial, including consent to translational research on FFPE and fresh frozen tumor biopsies in case the patient is enrolled into the trial.• The patient has been informed of and agrees to data transfer and handling, in accordance with national data protection guidelines.• Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1.• Life expectancy >3 months.• Hematopoietic status:<ul style="list-style-type: none">- Absolute neutrophil count $\geq 1.5 \times 10^9/L$,- Platelet count $\geq 100 \times 10^9/L$,- Hemoglobin ≥ 9 g/dL• Hepatic status:<ul style="list-style-type: none">- Serum total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN). In the case of known Gilbert's syndrome, a higher serum total bilirubin ($< 2 \times$ ULN) is allowed.- AST and ALT $\leq 2.5 \times$ ULN; if the patient has liver metastases, ALT and AST must be $\leq 5 \times$ ULN.• Renal status:<ul style="list-style-type: none">- Creatinine $\leq 1.5 \times$ULN or creatinine clearance > 60 mL/min- Proteinuria < 1 g/day• International Normalized Ratio (INR) or Prothrombin Time (PT) $\leq 1.5 \times$ ULN unless patient is receiving anticoagulant therapy as long as PT or PTT (partial thromboplastin time) is within therapeutic range of intended use of anticoagulant. <p><u>Exclusion criteria for screening:</u></p> <ul style="list-style-type: none">• Prior therapy with other anti-PD-1, anti- PD-L1, L2 or anti-CTLA4 therapy.• No FFPE material to centrally assess HER2-positivity and PD-L1 expression.• Known Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), or positive for Hepatitis B (HBsAg reactive) or Hepatitis C (HCV RNA [qualitative]).• Interstitial lung disease• History of or active pneumonitis requiring treatment with steroids
--	---



	<ul style="list-style-type: none">• Active central nervous system metastases, as indicated by clinical symptoms, cerebral edema, and/or progressive growth (patients with history of CNS metastases or spinal cord compression are eligible if they are clinically and radiologically stable for at least 4 weeks before first dose of investigational product and have not required high-dose steroid treatment in the last 4 weeks).• Leptomeningeal disease• History of clinically significant or uncontrolled cardiac disease, including congestive heart failure (New York Heart Association functional classification ≥ 3), angina, myocardial infarction or ventricular arrhythmia.• Previous severe hypersensitivity reaction to treatment with another monoclonal antibody.• Active infection requiring systemic therapy.• Chronic systemic therapy with immunosuppressive agents including corticosteroids.• Concurrent disease or condition that would make the patient inappropriate for trial participation or any serious medical disorder that would interfere with the patient's safety.• Known history of uncontrolled hypertension ($\geq 180/110$), unstable diabetes mellitus, dyspnea at rest, or chronic therapy with oxygen.• Dementia, altered mental status, or any psychiatric condition that would prevent the understanding or rendering of Informed Consent.• Treatment with an investigational agent in the 4 weeks before enrollment.• Active autoimmune disease or a documented history of autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Patients with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Patients that require intermittent use of bronchodilators or local steroid injections would not be excluded from the trial. Patients with hypothyroidism stable on hormone replacement or Sjögren's syndrome will not be excluded from the trial.• Chemotherapy, radioactive therapy, and/or biological cancer therapy within 3 weeks prior to the first trial dose and has not recovered to CTCAE v.4.0 grade 1 or better from adverse events.• Pregnant or lactating women; lactation has to stop before enrollment.• The patient of childbearing potential who is unwilling to use highly effective contraception during treatment and up to 7 months after
--	---



	<p>stop of trial treatment. Acceptable methods are intrauterine devices (without hormones), bilateral tubal occlusion, vasectomized partner or total abstinence. Oral, injectable, or implant hormonal contraceptives are not allowed.</p> <ul style="list-style-type: none">• Unresolved or unstable, serious adverse events from prior administration of another investigational drug.• Active or uncontrolled infection CTCAE v.4.0 grade 2 or higher.• Live vaccines within 30 days prior to the first dose of trial therapy and during trial treatment. <p><u>Inclusion criteria for enrollment:</u></p> <p>All inclusion criteria for screening, plus:</p> <ul style="list-style-type: none">• Central lab confirmation on a metastatic biopsy (or biopsy from unresectable loco-regional disease) of:<ul style="list-style-type: none">- HER2-positivity (immunohistochemistry score 3+) or ERBB2-amplification (Ratio ERBB2/centromeres ≥ 2.0 or mean gene copy number ≥ 6),- Presence of PD-L1 expression assessed by IHC (During the phase II portion of the trial a parallel, secondary cohort of 15 patients with PD-L1 negative disease will be enrolled see note in section 7.3.1).• Patient agrees to submit tumor tissue for translational research:<ul style="list-style-type: none">- tissue biopsy from unresectable loco-regional or metastatic disease obtained ≤ 1 year prior to enrollment or new tissue material from a recently obtained surgical or diagnostic biopsy. For patients who have presented with stage 4 disease de novo, a biopsy taken from the presumed primary breast lesion is acceptable (provided this was taken ≤ 1 year prior to enrollment).- if available: FFPE tumor block from primary surgery or diagnostic biopsy.- if available: pre-treatment fresh frozen tumor biopsy.- if feasible: FFPE tumor block from post-treatment biopsy will be taken at time of disease progression or end of all treatment if ended prior to progression. This re-biopsy is strongly advised.- if feasible: fresh frozen tumor biopsy from post-treatment biopsy will be taken at time of disease progression or end of all treatment if ended prior to progression.• Patient agrees to submit baseline (pre-treatment) blood and serial plasma for translational research, as detailed in Table 9 (see body of the protocol).• For patient of childbearing potential, negative serum pregnancy
--	--



	<p>test. Pregnancy test has to be repeated within 72h before treatment start.</p> <ul style="list-style-type: none"> • All anti-cancer treatment including endocrine therapy, with the exception of trastuzumab, must stop 3 weeks prior to first dose of trial treatment. <p><u>Exclusion criteria for enrollment:</u></p> <p>All exclusion criteria for screening apply for enrollment as well. Excluded are especially patients who have received any of the treatments below:</p> <ul style="list-style-type: none"> • Live vaccines within 30 days prior to the first dose of trial therapy and during trial treatment. • History of CNS metastases or spinal cord compression if they have not been clinically stable for at least 4 weeks before first dose of investigational product and require high-dose steroid treatment. • Treatment with an investigational agent in the 4 weeks before enrollment. • Patient has not recovered to CTCAE v.4.0 grade 1 or better from adverse events of prior therapy, except alopecia grade 2. • Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), or positive for Hepatitis B (HBsAg reactive) or Hepatitis C (HCV RNA [qualitative]).
<p>TRIAL OBJECTIVES AND ENDPOINTS</p>	<p><u>Primary objective</u></p> <p>The primary objectives of this phase Ib/II study are to determine the recommended dose of the <u>anti-PD-1 mAb, MK-3475</u>, in combination with standard dose trastuzumab, and to evaluate the efficacy and safety profile of the <u>drug combination</u> in patients with PD-L1 expressing, HER2-positive, unresectable loco-regional or metastatic breast cancer who have experienced progression during prior trastuzumab-based therapy.</p> <p><i>Note: The phase Ib part of the trial was completed in August 2015.</i></p> <p>The secondary objective is to explore the efficacy and safety of the drug combination in patients with PD-L1 negative, HER2-positive unresectable loco-regional or metastatic breast cancer who have experienced progression during prior trastuzumab-based therapy.</p> <p>Primary endpoint</p> <p>Phase Ib (dose finding): Dose-limiting toxicity of the anti-PD-1 mAb MK-3475 in combination with standard dose trastuzumab (defined in section 10.2.1)</p> <p>Phase II: Objective response (confirmed CR or PR as best overall response) based on RECIST 1.1 criteria (defined in section 13)</p>



	<p>Secondary endpoints</p> <p>For a precise definition of secondary endpoints please see section 17.1:</p> <ul style="list-style-type: none"> • Safety and tolerability as documented according to CTCAE v.4.0 • Disease control (DC) • Duration of response (DoR) • Time to progression (TTP) • Progression-free survival (PFS) • Overall survival (OS) <p>Correlative objectives</p> <p>Correlative studies will investigate:</p> <ul style="list-style-type: none"> • Responses according to levels of PD-L1, measured by IHC in metastatic (or unresectable loco-regional) lesion. • Responses according to levels of tumor infiltrating lymphocytes. • Responses according to ER status. • Responses according to FISH ratio and HER2 copy number. • Tumor dynamics during the disease course as well as emergence of new clones (i.e., resistance mechanisms) by determination of circulating plasma DNA (cpDNA). • Sequencing of material from tumor biopsies to determine molecular characteristics profiles of responders and non-responders <p>Exploratory objectives</p> <ul style="list-style-type: none"> • To describe TTP for patients with moderate partial response (MPR) compared with very good partial response (VGPR). • To explore the prognostic ability of pre-treatment ER, PD-L1, and TILs with respect to MPR and VGPR.
<p>TRANSLATIONAL RESEARCH</p>	<p>This is a proof-of-concept trial exploring a new therapeutic area for breast cancer. A complete collection of biological materials is requested in order to be able to comprehensively define the molecular and immune landscape of these tumors. The goals of the translational research will be to determine mechanisms and biomarkers for MK-3475 efficacy and resistance.</p> <ul style="list-style-type: none"> • Paired metastatic biopsies (FFPE +/- fresh frozen) at screening prior to enrollment and at progression after trial treatment for PD-L1 expression assessment and evaluation of tumor infiltrating lymphocytes. <ul style="list-style-type: none"> - Quantification of PD-L1 expression will be done by a certified IHC test in a central lab. - Quantification of tumor infiltrating lymphocytes will be



	<p>performed independently by two pathologists.</p> <ul style="list-style-type: none"> - Frozen and FFPE biopsies will be retained for sequencing using next generation technologies at the completion of the trial. • A whole blood sample to be taken prior to the start of trial treatment for future research purposes. This should be taken, processed and stored at -80°C anytime prior to start of study treatment. • Serial plasma samples for the characterization of circulating plasma DNA (cpDNA). <p>Translational research proposals not outlined in this protocol will be assessed by the IBCSG Biological Protocols Working Group for merit and feasibility.</p> <p>Biomarkers that are published in the future and considered to be of relevance can then also be assessed in the context of this trial.</p>
<p>STATISTICAL CONSIDERATIONS</p>	<p>Phase Ib: A standard “3+3” dose escalation design will be used to establish the RP2D of MK-3475 in combination with standard-dose trastuzumab. Two MK-3475 body weight based dose levels, and a fallback dose, will be tested.</p> <p>Phase II: PD-L1 expressing: A Simon's two-stage design will be used. The null hypothesis that the true objective response rate is 7% will be tested against a one-sided alternative of 22%. In the first stage, 17 patients will be enrolled. If there are 1 or fewer responses in these 17 patients, enrollment will be stopped. Otherwise, 23 additional patients will be accrued for a total of 40. The null hypothesis will be rejected if a total of 6 or more objective responses are observed in 40 patients. This design yields a type I error rate of 0.05 and power of 85% when the true objective response rate is 22%.</p> <p>PD-L1 negative: A single-stage design with an enrollment of 15 patients will be used to compare a null response rate of 1% with a desirable response rate of 20%. The decision rule is based on zero responses: the drug combination would not be considered worthy of further investigation if no patients have objective response.</p>
<p>NUMBER OF PATIENTS</p>	<p>This trial will enroll between 6 and 61 patients.</p>
<p>DURATION OF TRIAL</p>	<p>Assuming that the accrual rate will be approximately 4 patients per month, the recruitment will be completed within approximately 12-18 months after a start-up period of 6 months as the trial is activated by participating Centers. Clinical visits will end approximately 3 years after enrollment of the first patient, and sites will be contacted for a final update on survival status of the patients.</p>





2. Trial schedule

(See section 14 for detailed examinations schedule)

	≤3 months prior to treatment start ¹⁵	≤42 days prior to treatment start ¹⁵	≤28 days prior to treatment start ¹⁶	Day 1 of every 21 days cycle	Every second cycle: day 1 of cyc 3, 5, 7...	Weeks 12, 18, 24, 36...	Within 30 days after end of treatment	Follow-up 12 & 24 weeks prior PD ⁷	At progression	
Informed consent			x							
Medical history			x							
Physical examination, vital signs ¹ , weight, ECOG PS			x	x			x	x		
Serum pregnancy test ²			x							
Baseline symptoms and AEs ³			x							
Adverse events ³				x			x	x		
Concomitant medications		continuously.....						x	
Central HER2 and PD-L1 ⁴			x							
Treatment										
MK-3475				x						
Trastuzumab				x						
Laboratory tests										
Complete blood count ⁵			x	x			x			
Chemistry ⁶			x	x			x			
T3, T4, TSH		x ¹⁵			x		x			
Coagulation profile		x ¹⁵			√		x			
Hepatitis B and C, HIV		x ¹⁵								
CA 15-3			x		x		x			
Urinalysis		x ¹⁵			x					
Tumor Evaluation										
Clinical ⁷			x			x		x	x ⁸	
CT or MRI ⁷			x			x	x ¹	x	x ⁸	
Bone scan ⁷			√			√		√	x ⁸	
Cardiac evaluation⁹										
ECG	x ¹⁵					x		x		
Echocardiography or MUGA scan	x ¹⁵					x		x		
Cardiac enzymes			x			x				
Biological samples										
Metastatic FFPE biopsy ¹⁰			x						x	
Metastatic fresh frozen biopsy ¹¹			x						x	
Prim. surg. FFPE biopsy ¹²			x							
Blood sample ¹³			x							
Blood plasma ¹⁴		 see footnote 14							

x = mandatory √ = if medically indicated

Legend for trial schedule:

1. Vital signs include blood pressure, heart rate, respiratory rate and temperature. Blood pressure should be measured in both arms after sitting for 5 minutes. Weekly self- monitoring at home is recommended.



2. Pregnancy test for patient of childbearing potential (i.e., who are not yet menopausal and with their last period less than 12 months previously, no surgery to occlude the fallopian tubes, ovaries and/or womb not surgically removed). Test has to be repeated before treatment start, if treatment does not start within 72 hours of the previous test.
3. Baseline symptoms and adverse events should be recorded from signature of informed consent to prior to start of treatment. Adverse events should be reported at end of every cycle, at end of treatment visit and up to **30 days after stop of trial treatment**. Serious adverse events and pregnancies should be collected for 90 days following the end of treatment.
4. HER2 and PD-L1 must be determined centrally on an FFPE biopsy of either the archival metastatic tumor or a re-biopsy of a metastatic lesion (or unresectable loco-regional disease).

Laboratory tests

5. Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count).
6. Biochemistry: serum potassium, sodium, calcium, serum amylase, lipase **and LDH**;
Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;
Kidney function tests: urea, creatinine and uric acid.

At baseline: All labs need to be repeated within 7 days prior to first dose (not on day 1).

Subsequent treatment cycles: All labs need to be performed within 3 days prior to next dose.

Tumor evaluation

7. Clinical and radiological tumor assessments will be performed by CT scan or MRI at baseline, at 12, 18 and 24 **weeks (+/- 1)**, then every 12 **(+/- 2)** weeks until progression or a maximum of **24 weeks** after stop of treatment. **Patients who have received 24 months of trial treatment will have no further evaluations after the End of Treatment visit.** Bone scan will be performed if clinically indicated at the same time points. CR, PR or PD need to be confirmed with second assessment 4-6 weeks after first assessment. CT/MRI images must be stored (preferably in electronic format) at the sites for potential central review.
8. Disease progression needs to be confirmed with repeat imaging 4 to 6 weeks later.

Cardiac evaluation

9. All cardiac evaluations will be done at baseline, at weeks 12, 24, 36, etc. (every **12 +/- 2** weeks).

Biological studies (see section 15)

10. Two formalin-fixed, paraffin-embedded (FFPE) tumor blocks, one from the diagnostic biopsy of an unresectable loco-regional or metastatic lesion (preferably obtained in the screening phase, otherwise from evaluation of metastatic disease ≤ 1 year prior to enrollment) and one from a re-biopsy at progression (or end of trial treatment if prior to progression) must be submitted for central pathology review and stored in the IBCSG Tissue Bank for future translational research studies. This re-biopsy is strongly encouraged wherever feasible, except in cases of complete response (or minimal disease left) or or cases where too dangerous for the patient or impossible. If central evaluation at screening of archival tissue from a previous biopsy yields a negative test results, a new biopsy may be taken and submitted for central evaluation. **For patients who have presented with stage 4 disease de novo, a biopsy taken from the presumed primary breast lesion is acceptable, provided that it was taken ≤ 1 year prior to enrollment.**
11. When taking a biopsy, a fresh frozen (for RNA extraction) should be planned as well.
12. If the patient had **primary** surgery with curative intent, one tumor block from resection should be submitted.
13. Prior to first dose of MK-3475, EDTA whole blood sample for confirmation of somatic nature of mutations found in tumor samples.
14. For determination of circulating plasma DNA: plasma sample to be taken before the administration of the next dose of trial treatment at the start of cycles 1, 3, 5, 8 etc. (every 3 cycles thereafter), and **within 30** days after end of trial treatment (**corresponding with End of Treatment visit**). See section 15.1 for details.
15. **If taken as routine examination prior to signature of informed consent, and within the delays indicated (<3 months / ≤ 42 days), then they are acceptable for screening. HIV test prior to enrollment. All examinations taken specifically for screening purposes have to be done after signature of informed consent. Results of the examinations should be available at the time of registration of a new patient.**
16. **Informed consent may be signed before the ≤ 28 days period to facilitate the screening process.**



3. List of abbreviations

ADCC	Antibody dependent cellular cytotoxicity
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
BNP	B-type natriuretic peptide
CBR	Clinical benefit rate
CCL2	Chemokine (C-C motif) ligand 2
CD8/28/	Cluster of differentiation 8 / 28 / ..
CE	Cardiac events
CISH	Chromogenic in situ hybridization
cpDNA	Circulating plasma DNA
CR	Complete response
CRF	Case report form
CT	Computed tomography
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DC	Disease control
DILI	Drug-induced liver injury
DLT	Dose-limiting toxicity
DMC	Data Management Center
DoR	Duration of response
DSMC	Data Safety Monitoring Committee
EC50	Half maximal effective concentration
ECG	Electrocardiogram
ECI	Event of clinical interest
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
ER	Estrogen receptor
EU	European Union
FCGR-2A/3A	Low affinity immunoglobulin gamma Fc region receptor gene 2A or 3A
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence in situ hybridization
GCP	Good clinical practice
GnRH	Gonadotropin-releasing hormone
H&E	Hematoxylin and eosin stain
HER2	Human Epidermal growth factor Receptor 2
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
IC	Informed consent
ICH	International Conference on Harmonization
iDF	iDataFax
IFNγ	Interferon gamma
IHC	Immunohistochemistry
IL	Interleukin



IMP	Investigational Medicinal Product
ITSM	Immunoreceptor tyrosine-based switch motif
irAE	Immune-related adverse event
LPBC	Lymphocyte-predominant breast cancer
LVEF	Left ventricular ejection fraction
mAb	Monoclonal antibody
MPR	Moderate partial response
MRI	Magnetic resonance imaging
MSD	Merck Sharp & Dohme Corp.
MTD	Maximum tolerated dose
MUGA	Multi gated acquisition -scan
CTCAE	Common toxicity criteria for adverse events
NK-cells	Natural killer cells
NSCLC	Non-small cell lung cancer
NYHA	New York Heart Association
ORR	Overall response rate
PD	Progressive disease
PD-1	Programmed death receptor 1
PD-L1	Programmed death receptor 1 ligand
PET	Positron emission tomography
PFS	Progression free survival
PgR	Progesterone receptor
PI	Principal Investigator
PIS/IC	Patient information sheet / informed consent
PK	Pharmacokinetics
PR	Partial response
PS	Performance status
Q3W	Every 3 weeks
RCC	Renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended phase II dose
SAE	Serious adverse event
SD	Stable disease
SERM	Selective estrogen receptor modulator
SNP	Single nucleotide polymorphism
SPC	Summary of Product Characteristics
T3	Triiodothyronine
T4	Thyroxine
T-DM1	trastuzumab-emtansine
TIL	Tumor infiltrating lymphocyte
TSH	Thyroid Stimulating Hormone
TTP	Time to progression
ULN	Upper limit of normal
VGPR	Very good partial response



4. Background and scientific rationale

4.1. HER2-positive metastatic breast cancers

The development of therapies targeted against HER2 antibodies has dramatically changed the natural history of HER2/neu overexpressing (or HER2-positive) breast cancer. Trastuzumab is a monoclonal antibody (mAb) targeting human epidermal growth factor receptor 2 (HER2). In the pivotal trial, Slamon et al [1] have reported that adding trastuzumab to paclitaxel improves both progression free and overall survival. Since then, trastuzumab has become the standard of care for patients presenting HER2-positive metastatic breast cancer. Large phase III trials in the adjuvant setting have confirmed that its addition to standard cytotoxic chemotherapy has significantly improved clinical outcomes for women with this once devastating disease [2].

Recently, there have been some significant advances in the treatment of HER2-positive disease. The use of combination targeted anti-HER2 therapies have been shown to improve outcome of patients with HER2-positive metastatic breast cancer. These therapies include lapatinib [3], pertuzumab [4] and trastuzumab-emtansine (T-DM1) [5], the latter two therapies were recently approved by the US Food and Drug Administration (FDA). T-DM1 has been shown to improve progression-free survival as compared with the combination of trastuzumab and docetaxel. In addition, pertuzumab has been shown to improve outcome when added to a trastuzumab-docetaxel regimen in the first line metastatic setting. Recent developments suggest that the combination of T-DM1 and pertuzumab will further improve outcome and become standard of care in the first line setting [6]. Lapatinib is currently being evaluated in the adjuvant setting in combination with trastuzumab and in patients who develop brain metastases. Nevertheless, despite all these advances, the majority of patients presenting with stage IV HER2-positive breast cancer will ultimately become resistant to these agents and die of their disease. Thus, there is still a need to develop new therapeutic agents for patients presenting with HER2-positive metastatic breast cancer.

4.2. Rationale for combining immune checkpoint modulators and trastuzumab

Despite the significant improvements in clinical outcome for women with HER2 overexpressing disease, there is still a need to improve therapy and to better identify women that may respond best to such treatments. There are currently no prognostic biomarkers that can help physicians identify the women with HER2-positive disease that are resistant to trastuzumab in addition to standard chemotherapy. This may be due to the fact that despite a significant amount of research, the dominant mechanism of trastuzumab's efficacy is still debatable. Signaling blockade is important but antibody dependent cellular cytotoxicity (ADCC) via interactions with Fcγ receptors on leucocytes has also been implicated as an important mechanism of action as mice that lack Fcγ receptors do not respond to trastuzumab. Variations of single nucleotide polymorphisms (SNPs) in FCGR3A and FCGR2A genes that affect binding of antibodies to Fcγ receptors in 1286 patients were not associated with lack of trastuzumab efficacy [2].



Whilst breast cancer has traditionally not been considered “immunogenic” when compared to disease such as melanoma and renal cell carcinoma, increasing data suggests the contrary. For example, tumor infiltrating lymphocytes (TILs) seen in the breast cancers at the time of diagnosis have been associated with clinical outcome and therapeutic responses. Infiltrating lymphocytes have been associated with a good prognosis in a number of other solid tumor types. Recently large cohort studies have confirmed this association also for breast cancer, in particular, for HER2-positive and triple negative breast cancer subtypes [3-5]. HER2-positive and triple negative breast cancers also have significantly higher levels of lymphocytic infiltration at diagnosis, as compared with tumors that are of the luminal subtype (ER+/HER2-).

Prognostic gene expression profiling studies in primary breast cancer also support this concept and have consistently shown an association between high expression of immune-related genes and a good prognosis in HER2-positive disease. The analysis of microarray data from thousands of patients has shown that a high expression of a *STAT1* metagene is strongly associated with better prognosis in HER2-positive disease [7-10]. Interrogating public microarray data also illustrates that significantly higher levels of PD-L1 (CD274) are seen in HER2-positive breast cancers (unpublished data, $p < 0.0001$). Higher levels of PD-L1 expression have been associated with poor clinic-pathological characteristics in breast cancer [11].

As high levels of the *STAT1* signature were correlated with increasing lymphocytic infiltration on tumor Hematoxylin and Eosin stain (H&E) sections, TILs were examined as a potential biomarker of response to trastuzumab in HER2-positive disease. Samples from a phase III adjuvant clinical trial that randomized HER2-positive breast cancer patients to trastuzumab vs. no trastuzumab were analyzed to determine the association between the amount of lymphocytic infiltration in the tumor and the degree of trastuzumab benefit. It was reported that higher levels of lymphocytic infiltrate was associated with a higher magnitude of benefit from trastuzumab treatment in addition to cytotoxic chemotherapy [12]. This data also suggested that a pre-existing immune response seemed to be necessary for trastuzumab efficacy and that trastuzumab may release immunosuppression induced by the tumor microenvironment through one or more yet to be defined mechanisms.

Recent preclinical data have reported that tumor regressions from anti-HER2 therapy in a preclinical mouse model require both effective innate and adaptive immunity [13, 14]. As proof-of-concept, enhancement of T-cell responses in combination with trastuzumab was evaluated in an immunocompetent transgenic HER2 mammary mouse model. In this model, the combination of the anti-HER2 mAb and anti-PD-1 therapy was synergistic and more effective than either monotherapy [14]. These data suggest that HER2-overexpressing tumors use immunosuppression as a mechanism to facilitate growth and progression.

The concept that oncogene inhibition and immune modulation cooperate has been demonstrated preclinically in other solid cancer types. For example, in melanoma, it has been reported that PLX4720 treatment can downregulate tumor CCL2 gene expression and decrease tumor CCL2 expression in both BrafV600E mouse melanoma transplants and in de novo melanomas in a manner that was coincident with reduced tumor growth. These data



implicate host CCL2 in the mechanism of action of type I BRAF inhibitors and support the therapeutic potential of combining BRAF inhibitors with immunotherapy [15]. A similar concept has been recently reported that links efficacy of the tyrosine kinase inhibitor therapy, imatinib, in gastrointestinal stromal tumor (GIST), with reduction of tumor expression of IDO1 [16-18]. Interestingly, in this situation, imatinib has been shown to promote both T-cell and natural killer (NK) cell responses in mice and humans, respectively, while both NK-cells and CD8+ T-cells have been implicated in the activity of a trastuzumab-like molecule in the mouse [19]. Furthermore, Rakhra and colleagues have shown that an intact immune system is required for tumor regressions related to inhibition of the driving oncogene in “oncogene addicted” cancers [19]. Host immunity was shown to participate in cellular senescence, angiogenesis decrease and release of chemokines, which ultimately leads to tumor regressions on withdrawal of the driving oncogene. Together these data suggest that oncogene addiction mechanisms can also suppress anti-tumor immunity.

Together, the preclinical and clinical data suggest that one of trastuzumab’s main mechanisms of action is through facilitating functional anti-tumor immunity in HER2-positive breast cancers. This background provides the rationale for the present trial, which will evaluate if patients with HER2-positive disease will benefit from therapies that modulate the immune environment.

4.3. **Anti-PD-1 and PD-L1 antibodies**

The importance of immunity in controlling tumor initiation, progression and growth has been speculated on for decades. In general, tumors must learn to evade and avoid immune surveillance and destruction. This is supported by a higher frequency of cancer incidence in transplant patients on immunosuppression. However, whilst there is no increase in breast cancer incidence in transplant compared with the normal population, suggesting that immunosurveillance does not regulate the initiation of primary breast tumors, outcomes seem to be worse [20]. Data have shown that immunosurveillance is important in regulating breast cancer metastases and that pathways that control metastases have effects independent of the primary tumor [21-23].

The term immune evasion refers to the ability of the tumor to suppress and change host anti-tumor immune reactions. The programmed cell death 1 (PD-1) pathway represents a major immune control switch which may be engaged by tumor cells to overcome active T-cell immune surveillance [24]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in various tumors [25-28]. High expression of PD-L1 on tumor cells (and to a lesser extent of PD-L2) has been found to correlate with poor prognosis and survival in various other solid tumor types, including renal cell carcinoma (RCC) [29], pancreatic carcinoma [30], hepatocellular carcinoma [31], ovarian carcinoma [32] and non-small cell lung cancer (NSCLC) [33]. Furthermore, PD-1 has been suggested to regulate tumor-specific T-cell expansion in patients with malignant melanoma [34]. This observed correlation suggests that the PD-1/PD-L1 pathway plays a critical role in the tumor immune evasion and could be considered an attractive target for many solid organ types.



The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [35, 36]. The structure of murine PD-1 has been resolved [37]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 ζ , PKC θ and ZAP70 which are involved in the CD3 T-cell signaling cascade [36, 38-40]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [41]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4⁺ and CD8⁺ T-cells, B-cells, regulatory T-cells (Tregs) and NK-cells [42, 43]. Expression has also been shown during thymic development on CD4-CD8⁻ (double negative) T-cells as well as subsets of macrophages and dendritic cells [44]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [25, 26, 28]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [27]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor.

The observed correlation of clinical prognosis with PD-L1 expression in multiple cancers suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8⁺ T-cells and leads ultimately to tumor rejection, either as a monotherapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a monotherapy in models of squamous cell carcinoma, pancreatic carcinoma, melanoma and colorectal carcinoma.



Recent data of nivolumab (MDX-1106, BMS-936558), an IgG4 antibody against PD-1, have validated PD-1 as an attractive target for clinical therapeutic intervention. In a recent report of the clinical trial data of nivolumab a total of 296 patients with advanced NSCLC, castration resistant prostate cancer, or RCC or colorectal cancer were treated at a dose of 0.1, 0.3, 1, 3, or 10 mg/kg every 2 weeks. Among 236 evaluable patients, cumulative response rates (all doses) defined by the Response Evaluation Criteria in Solid Tumors [RECIST] were 18% among patients with NSCLC, 28% among patients with melanoma, and 27% among patients with RCC. Responses were reported to be durable; 20 of 31 responses lasted 1 year or more in patients with 1 year or more of follow-up. The most common adverse events were fatigue, decreased appetite, diarrhea, nausea, cough, dyspnea, constipation, vomiting, rash, pyrexia, and headache. Grade 3 or 4 adverse events were observed in 49% of patients, while 14% of patients had treatment-related Grade 3 or 4 adverse events. Drug-related adverse events of special interest (e.g., those with potential immune-related causes) occurred in 41% of patients and included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis. There were three deaths from treatment related pneumonitis, two in NSCLC and one in colorectal cancer patients. No maximum tolerated dose was defined for nivolumab [33, 45]. Interestingly, PD-L1 expression was highly predictive for the efficacy of anti-PD-1 antibodies. In the PD-L1-non expressing population, no response was observed. At the opposite, 9 out of 25 patients with PD-L1 expressing cancer had presented an objective response.

MK-3475 (previously known as SCH 900475) is a potent and highly selective humanized mAb of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Both MK-3475 and nivolumab contain the S228P stabilizing mutation and have no antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) activity. MK-3475 strongly enhances T-lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. In T-cell activation assays using human donor blood cells, the EC50 was in the range of 0.1 to 0.3 nM. MK-3475 also modulates the level of interleukin-2 (IL-2), tumor necrosis factor alpha (TNF α), interferon gamma (IFN γ), and other cytokines. The antibody potentiates existing immune responses only in the presence of antigen and does not nonspecifically activate T- cells.

Keytruda™ (pembrolizumab, MK-3475) was approved in the United States for the treatment of patients with melanoma in September 2014. In 2015, it was approved in Australia, the European Union (EU), as well as in several other countries. Recently, the drug was approved in the US for the treatment of non-small cell lung cancer.

Overall, these data suggest that anti PD-L1 / PD-1 antibodies present antitumor activity in PD-L1 expressing cancers. This drug family has not been tested in breast cancer patients. Preclinical and correlative data suggests that HER2-positive breast cancer could be particularly amenable to immunotherapeutic approaches. We therefore propose to evaluate if a monoclonal antibody targeted against PD-1, a T-cell negative regulator, can reverse trastuzumab resistance in patients having previously progressed on trastuzumab.



4.3.1. Preliminary efficacy data in breast cancer

The KEYNOTE-012 study was a phase Ib study of pembrolizumab (MK-3475) in patients with advanced triple-negative breast cancer [46]. In this phase 1b study, 32 patients with metastatic triple negative breast cancer were treated at a dose of 10 mg/kg intravenously every 2 weeks. Patients were required to have a PD-L1 positive tumor, defined as any immunohistochemical staining in the tumor stroma or on greater than or equal to 1% of tumor cells. The rate of PD-L1 positivity was 58% in the screening population. Over 90% of the enrolled patients were previously treated with taxane, anthracycline, capecitabine or platinum based chemotherapy, with 21 of 32 having had more than 1 line of therapy. 56.3% patients experienced a treatment related adverse event of any grade. Grade 3 treatment-related events consisted of anemia, headache, aseptic meningitis and pyrexia. One patient developed disseminated intravascular coagulation that resulted in death, which was attributed to treatment. The median time on therapy with MK-3475 was 59.5 days. The overall response rate by RECIST 1.1 was 18.5% (5/27 patients evaluable), with 1 complete response and 4 partial responses. Of the 5 responding patients, 4 had received greater than 2 lines of prior chemotherapy. Three patients did not receive a post-baseline response evaluation due to rapid progression. Responding patients continued on therapy for 40 weeks or more, with the median duration of response not reached at the time of reporting. The authors noted that due to the small number of patients, they were unable to establish whether higher levels of PD-L1 positivity were associated with improved response. A phase II study is planned for 2015 (KEYNOTE-086, NCT02447003).

Another phase Ia study used an anti-PD-L1 antibody MPDL3280A in patients with PD-L1 positive triple negative breast cancers [47]. PD-L1 on tumor infiltrating immune cells was assessed using a proprietary immunohistochemistry assay developed by the trial sponsor. The presence of PD-L1 on greater than or equal to 5% of tumor infiltrating immune cells was considered a positive result. Of the 21 evaluable patients, the overall response rate was 19%. Three cases of pseudo-progression were also observed. Amongst the screening population, the number of PD-L1 positive patients was 23%. Based on this promising data, a phase III study is ongoing (NCT02425891). This study will randomize newly diagnosed patients with advanced triple negative breast cancer to abraxane +/- MPDL3280A. Notably, patients will be unselected for PD-L1 positivity.

4.3.2. Rationale for the PD-L1 negative cohort

Lately MK-3475 has been evaluated in NSCLC independent of PD-L1 expression. In KEYNOTE-001, the response rate for all NSCLC patients was ~23%; high PD-L1 positivity ($\geq 50\%$) was associated with increased ORR (45%), but patients with PD-L1-negative ($< 1\%$) tumors also showed responses (ORR ~9-12%) [48].

In patients for whom PD-L1 staining using the clinical trial IHC assay was available, the objective response rate (ORR), PFS (hazard ratio, HR, 0.52), and OS (HR, 0.59) were higher in patients with strong PD-L1 expression ($\geq 50\%$ staining) than in patients with weak/negative PD-L1 expression [48].



Across studies conducted thus far it appears that patients whose tumors express PD-L1, as detected by the various IHC assays, have numerically higher responses to PD-1/PD-L1 blockade than those who do not. However, durable and long lasting responses have also been observed in solid tumor types without PD-L1 expression and these patients should also be considered eligible for PD-1/PD-L1 blocking agents [33, 49-51]. Also, in a recently conducted meta-analysis of 20 trials (1,475 patients) with nivolumab, pembrolizumab, and atezolizumab in NSCLC, melanoma, and genitourinary cancers, correlations between the tumor PD-L1 expression and response were investigated. Significant differences were seen in NSCLC and melanoma, but not genitourinary cancers. Ignoring the different cutoffs for PD-L1 expression in different trials, responses to nivolumab and pembrolizumab were observed in 34.1% and 19.9% of patients with PD-L1-positive and PD-L1-negative tumors, respectively. This difference was statistically significant, but also showed that absence of tumor PD-L1 expression is not a good enough biomarker to exclude patients from receiving an anti-PD-1 drug, as such patients may still derive benefit from PD-1 blockade [52].

It remains unclear if the differential expression or the expression on infiltrating lymphocytes vs. tumor will account for the differences in response rates seen and how this will vary by tumor type. Further research is needed to understand the technical limitations of the test, tumor heterogeneity and the significance of varying degrees and location of PD-L1 expression. As such, current recommendations are that patients should not be selected for treating according to PD-L1 status and thus this forms the rationale for an inclusion of a PD-L1 negative expression cohort in this study [53].

4.4. Trial hypothesis

This is a proof-of-concept clinical trial to evaluate the hypothesis that the combination of an immune re-activation approach and anti-HER2 therapy can reverse trastuzumab resistance and improve clinical outcomes in HER2-positive disease. The trial will investigate the efficacy of an anti-PD-1 mAb in the setting of trastuzumab resistant, HER2-positive metastatic breast cancer. The aim of this trial is to demonstrate that immune reactivation by inhibition of PD-1 is associated with objective evidence of efficacy in this population. Patients included in this trial will have metastatic or incurable disease as it is essential to demonstrate the safety of the combination first in this setting.

While there are no overlapping toxicities between trastuzumab and MK-3475, a phase Ib run-in will be performed first to **evaluate the safety profile of the drug combination**. **Three doses of MK-3475 will be considered**: 1 mg/kg, 2 mg/kg and 10 mg/kg. These three doses have all been shown to be associated with tumor responses. For the phase II part, **the primary assessment of efficacy will be based on a single-arm trial design in a trastuzumab-resistant population with PD-L1-expressing disease**. **As a secondary objective, efficacy in patients with PD-L1 negative disease will be based on a parallel cohort, single-arm design**. As it is standard-of-care in the HER2-positive metastatic population to continue an anti-HER2 therapy back-bone even after progression, trastuzumab will be given to the patient in combination with MK-3475. Given that the immune mechanisms are unknown and may be quite different in combinations of trastuzumab plus pertuzumab, trastuzumab with taxanes and trastuzumab-emtansine (T-DM1), demonstration of proof-of-concept first with the



combination of trastuzumab alone plus MK-3475 was deemed essential prior to moving on to combination anti-HER2 therapies.

4.5. Overall risk-benefit assessment

The metastatic or advanced setting was chosen to evaluate this new therapy combination. The setting of incurable disease is acceptable to justify the evaluation of a potential new combination in breast cancer. As these patients are resistant to the standard therapy of trastuzumab, an investigational therapy that could potentially reverse this resistance is of high interest in this patient population. The available pharmacokinetics and pharmacodynamics data provide scientific rationale for exploring the 3-weekly schedule in combination with the 3-weekly trastuzumab dose. Safety will be carefully monitored throughout the trial.

4.6. Rationale for the trial design

A MK-3475 monotherapy arm was not considered ethical in this population as continued HER2-blockade is standard of care in this population. Supporting this, in a potential follow-on phase III trial, combinations with trastuzumab and other anti-HER2 will be used. As the current standard-of-care for this population is changing, with docetaxel, pertuzumab and trastuzumab receiving US FDA approval for the first line setting and trastuzumab-emtansine (T-DM1) (+/- pertuzumab) likely to be also registered in the next 2 years for this indication, women with previous exposure to these combinations will be eligible for this trial. As immunotherapy is yet to be tested in breast cancer patients, this trial will use a phase Ib/II approach to **evaluate** the **safety profile** of the combination of MK-3475 and trastuzumab and provide estimates of efficacy and tolerability.

4.6.1. Rationale for Dose Selection/Regimen/Modification

An open-label phase I trial (KEYNOTE-001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. In addition, two randomized cohort evaluations of melanoma subjects receiving MK-3475 at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed, and one randomized cohort evaluating 10 mg/kg Q3W versus 10 mg/kg Q2W has also been completed. The clinical efficacy and safety data demonstrate a lack of important differences in efficacy or safety profile across doses.

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



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

The rationale for further exploration of 2 mg/kg and comparable doses of MK-3475 in solid tumors is based on: 1) similar efficacy and safety of MK-3475 when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients; 2) the flat exposure-response relationships of MK-3475 for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W; 3) the lack of effect of tumor burden or indication on distribution behavior of MK-3475 (as assessed by the population PK model); and 4) the assumption that the dynamics of MK-3475 target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of MK-3475 showing that the flat dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks; 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response; and 3) will maintain individual patients' exposure in the exposure range established in melanoma that are well tolerated and safe.

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

5. Trial objectives and endpoints

The primary objectives of this phase Ib/II study are to determine the recommended dose of the anti-PD-1 mAb, MK-3475, in combination with standard dose trastuzumab, and to evaluate the efficacy and safety profile of the drug combination in patients with PD-L1 expressing, HER2-positive, unresectable loco-regional or metastatic breast cancer who have experienced progression during prior trastuzumab-based therapy.

Note: The phase Ib part of the trial was completed in August 2015.



The secondary objective is to explore the efficacy and safety of the drug combination in patients with PD-L1 negative, HER2-positive unresectable loco-regional or metastatic breast cancer who have experienced progression during prior trastuzumab-based therapy.

5.1. Primary endpoint

Phase Ib (dose finding): Dose-limiting toxicity of the anti-PD-1 mAb MK-3475 in combination with standard dose trastuzumab (defined in section 10.2.1)

Phase II: Objective response (confirmed CR or PR as best overall response) based on RECIST 1.1 criteria (defined in section 13).

5.2. Secondary endpoints

For a precise definition of secondary endpoints please see Section 17.1:

- Safety and tolerability as documented according to CTCAE version 4.0
- Disease control (DC)
- Duration of response (DoR)
- Time to progression (TTP)
- Progression-free survival (PFS)
- Overall Survival (OS)

5.3. Correlative objectives

Correlative studies will investigate:

- Responses according to levels of PD-L1, measured by IHC in metastatic (or unresectable loco-regional) lesion.
- Responses according to levels of tumor infiltrating lymphocytes.
- Responses according to ER status.
- Responses according to FISH ratio and HER2 copy number.
- Tumor dynamics during the disease course as well as emergence of new clones (i.e., resistance mechanisms) by determination of circulating plasma DNA (cpDNA).
- Sequencing of material from tumor biopsies to determine molecular characteristics profiles of responders and non-responders.

5.4. Exploratory objectives

5.4.1. To describe TTP for patients with moderate partial response (MPR) compared with very good partial response (VGPR).

5.4.2. To explore the prognostic ability of pre-treatment ER, PD-L1, and TILs with respect to MPR and VGPR.

6. Trial design, duration and termination



6.1. Trial design

This is a phase Ib/II, open-label, multi-center trial **with primary objectives** for patients with HER2-positive, PD-L1 expressing, unresectable loco-regional or metastatic breast carcinoma who have previously progressed on prior trastuzumab-based therapy. **During the phase II portion of the trial, a secondary cohort of 15 patients with PD-L1 negative disease will be enrolled.**

The phase Ib trial will use a standard 3+3 dose-escalation design to determine the RP2D of the anti-PD-1 mAb, MK-3475, in combination with standard dose trastuzumab using 3 possible **body weight based** dose levels. **The phase II portion of the trial will use a flat dose of 200 mg MK-3475 in combination with standard dose trastuzumab.**

Each patient will receive combination trastuzumab and anti-PD-1 mAb MK-3475 until disease progression, lack of tolerability, completion of 24 months **after start** of treatment with MK-3475, or patient declines further protocol treatment.

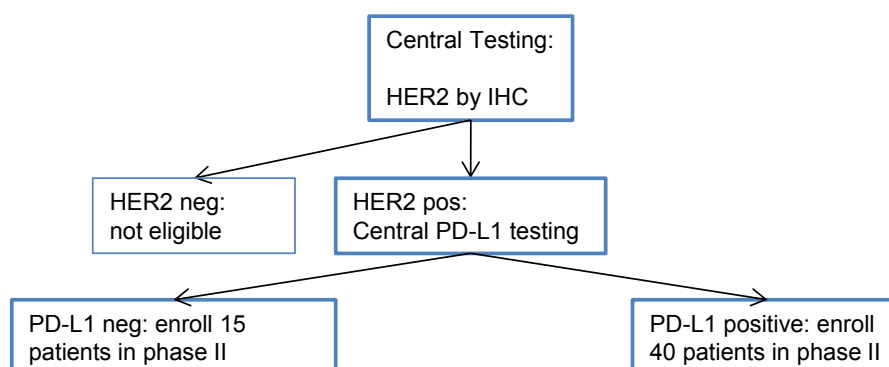
For the primary objectives, patients' disease must be confirmed to express PD-L1 based on an FFPE biopsy taken from an unresectable loco-regional or metastatic lesion using a certified IHC test in a designated lab. HER2 status of the tumor will also be centrally confirmed at the same time. Patients whose disease is both PD-L1 expressing and HER2-**positive** (IHC 3+ or FISH/chromogenic in situ hybridization [CISH] positive) are eligible to be enrolled. **For the secondary objective, a cohort of 15 patients with PD-L1 negative disease will be enrolled during the phase II portion of the trial.**

It is expected that a maximum of **61** evaluable patients will be enrolled.

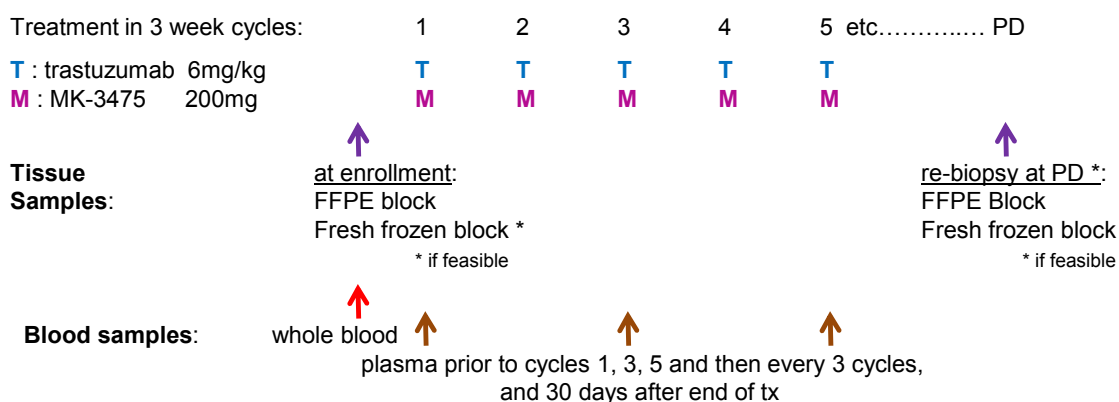


6.2. Trial schema

Screening: unresectable locoregional or metastatic breast cancer overexpressing HER2
 → Submit **an FFPE block from** core biopsy for central testing



Phase Ib: dose finding for MK-3475 in 3+3 design — **Phase II** 200mg



Section 8.1 describes central testing in the screening phase.

Treatment: The phase Ib dose-escalation part of the trial will determine the RP2D of MK-3475 from 3 possible **body weight based** dose levels based on the tolerability observed in cycle 1: trastuzumab 6 mg/kg and MK-3475 at 2 mg/kg, 10 mg/kg, or a fall-back dose of 1 mg/kg. The phase II, single-arm trial **will use a flat dose of 200 mg MK-3475 in combination with standard dose trastuzumab**. Patients will receive combination trastuzumab and anti-PD-1 mAb until progression, lack of tolerability, completion of 24 months of treatment with MK-3475, or patient declines further protocol treatment.

6.3. Clinical evaluations

Tumor response will be evaluated according to RECIST version 1.1 (see Section 13) using computed tomography (CT) or magnetic resonance imaging (MRI) of the thorax, abdomen and pelvis. For each patient the same technique must be used to evaluate each lesion through the trial.

- Tumor assessments will be performed at screening (prior to therapy), at 12, 18 and 24 **weeks (+/- 1)** and every 12 **(+/- 2)** weeks thereafter until progressive disease is



observed or for a maximum of **24 weeks** after treatment stop (see also Section 14.9).

- A response (CR or PR) must be confirmed with repeat imaging between 4 and 6 weeks later.

If the imaging shows progressive disease (PD), imaging must also be repeated between 4 and 6 weeks later in order to confirm PD.

Note: Patients deemed clinically unstable are not required to have repeat imaging for confirmation. It is at the discretion of the Investigator to keep the patient on trial treatment as long as PD has not been confirmed by a second imaging, or stop trial treatment immediately. The decision whether to continue trial treatment should be based on clinical judgment, including the patient's overall condition, performance status, symptoms and laboratory values. See detailed instructions in Section 10.9.

6.4. Biological evaluations

Biopsies taken pre-therapy are used to confirm HER2-positivity and PD-L1 expression **status**. New tissue material from a recently obtained surgical or diagnostic biopsy is preferred. A previous biopsy from an unresectable loco-regional or metastatic lesion obtained ≤ 1 year prior to enrollment may be used (i.e., prior to a previous line of therapy), however if this is negative for PD-L1, another biopsy may be taken (i.e., if during a period of the trial when only patients with PD-L1 expressing disease are being enrolled).

Collection of tumor tissue and repeat blood specimens is mandatory on this trial. Please see Section 15 for a complete overview of types of samples and collection times.

6.5. Sample size and trial duration

- The sample size for the phase Ib trial will be between 6 and 12 enrolled patients and will depend on the number of dose cohorts needed to determine the RP2D. A patient in the phase Ib portion of the trial will be replaced if determination of DLT cannot be adequately assessed because of rapid disease progression during the first cycle of therapy, or if treatment and/or follow-up is stopped during the first cycle of therapy for reasons other than toxicity.

Note: The phase Ib part of the trial was completed in August 2015 with 6 patients enrolled.

- The phase II trial will **enroll two parallel cohorts**. The primary cohort of patients with PD-L1 expressing disease will use a Simon two-stage design with a total target sample size of 40 patients. A secondary cohort of 15 patients with PD-L1 negative disease will also be enrolled, using a single-stage design.
- We estimate that the total sample size will be between 6 and **61** patients. The minimum would occur if the trial is stopped quickly in the phase Ib phase due to toxicity.
- The enrollment of **61** patients is expected to occur over a period of 12 to 18 months after a start-up period of 6 months as the trial is activated by participating Centers.



After cessation of treatment, patients will be followed in clinic until **documented disease** progression or **24 weeks** after stop of treatment, whichever occurs first. Clinical visits will therefore end approximately 3 years after enrollment of the first patient. Sites will be contacted for a final update on **subsequent therapy and** survival status of the patients.

The trial will be conducted in **11** Centers in 5 countries. Under the assumption that approximately **two thirds** of patients will not meet the inclusion/exclusion criteria, it is estimated that **around 120** patients will be screened to enroll **61** patients.

7. Patient selection

7.1. Eligibility criteria for screening

7.1.1. Female gender

7.1.2. Age ≥ 18 years

7.1.3. Histologically confirmed breast adenocarcinoma that is unresectable loco-regional, or metastatic.

7.1.4. Locally confirmed HER2-positivity (immunohistochemistry score 3+) or ERBB2-amplification (Ratio ERBB2/centromeres ≥ 2.0 or mean gene copy number ≥ 6) of primary tumor or of **biopsy from** metastatic or unresectable loco-regional **lesion**.

Note: Do not submit for central review if the metastatic or unresectable loco-regional biopsy tested **HER2-negative** locally.

7.1.5. Must have **trastuzumab resistant disease, defined by**

- demonstrated progression of disease while on treatment with trastuzumab (**monotherapy or combination-based therapy**) or **trastuzumab-emtansine (T-DM1)** for metastatic or unresectable loco-regional disease.
- **recurrence while on adjuvant trastuzumab or within 12 months of completing adjuvant trastuzumab.**
- **Any number of prior lines of anti-HER2 therapy acceptable. Patients for whom the treatment with the current first-line combination of trastuzumab, pertuzumab and taxanes is not an option (e.g., due to refusal or contraindication) can be considered for enrollment into this trial.**

Progression/**recurrence** must have been demonstrated by radiological or clinical assessment.

7.1.6. If a patient has received a subsequent anti-HER2 therapy, she must also have progressed on the subsequent therapy. Progression must have been demonstrated by radiological or clinical assessment.



- 7.1.7. Presence of at least one measurable lesion as defined by RECIST 1.1.
- 7.1.8. LVEF $\geq 50\%$
- 7.1.9. Patient agrees to submit an FFPE tumor biopsy for central confirmation of HER2-positivity and central assessment of PD-L1 status. This can be from archival tissue from unresectable loco-regional or metastatic disease obtained ≤ 1 year prior to enrollment or new tissue material from a recently obtained surgical or diagnostic biopsy. Tissue obtained for the biopsy must not have been previously irradiated.

Note: Central Pathology Review on a tumor biopsy is mandatory for this trial, and patients will be evaluated centrally for eligibility.

- 7.1.10. Written Informed Consent (IC) for screening procedures and trial participation must be signed and dated by the patient and the Investigator prior to screening.
- 7.1.11. Written consent to biological material submission, indicating the patient has been informed of and agrees to tissue and blood material use, transfer and handling, must be signed and dated by the patient and the Investigator prior to any procedures specific for this trial, including consent to translational research on FFPE and fresh frozen tumor biopsies in case the patient is enrolled into the trial.
- 7.1.12. The patient has been informed of and agrees to data transfer and handling, in accordance with national data protection guidelines.
- 7.1.13. Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1 (see below*).
- 7.1.14. Life expectancy >3 months.
- 7.1.15. Hematopoietic status:
- Absolute neutrophil count $\geq 1.5 \times 10^9/L$,
 - Platelet count $\geq 100 \times 10^9/L$,
 - Hemoglobin ≥ 9 g/dL
- 7.1.16. Hepatic status:
- Serum total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN). In the case of known Gilbert's syndrome, a higher serum total bilirubin ($<2 \times$ ULN) is allowed.
 - AST and ALT $\leq 2.5 \times$ ULN; if the patient has liver metastases, ALT and AST must be $\leq 5 \times$ ULN.
- 7.1.17. Renal status:
- Creatinine $\leq 1.5 \times$ ULN or creatinine clearance >60 mL/min
 - Proteinuria <1 g/day



7.1.18. International Normalized Ratio (INR) or Prothrombin Time (PT) $\leq 1.5 \times$ ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulant.

7.2. Exclusion criteria for screening

7.2.1. Prior therapy with other anti-PD-1, anti- PD-L1, L2 or anti-CTLA4 therapy.

7.2.2. No FFPE material to centrally assess HER2-positivity and PD-L1 expression.

7.2.3. Known Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), or positive for Hepatitis B (HBsAg reactive) or Hepatitis C (HCV RNA [qualitative]).

7.2.4. Interstitial lung disease

7.2.5. History of or active pneumonitis requiring treatment with steroids

7.2.6. Active central nervous system metastases, as indicated by clinical symptoms, cerebral edema, and/or progressive growth (patients with history of CNS metastases or spinal cord compression are eligible if they are clinically **and radiologically** stable for at least 4 weeks before first dose of investigational product and **have not required** high-dose steroid treatment **in the last 4 weeks**).

7.2.7. Leptomeningeal disease

7.2.8. History of clinically significant or uncontrolled cardiac disease, including congestive heart failure (New York Heart Association functional classification ≥ 3 , see Table 4), angina, myocardial infarction or ventricular arrhythmia.

7.2.9. Previous severe hypersensitivity reaction to treatment with another monoclonal antibody.

7.2.10. Active infection requiring systemic therapy.

7.2.11. Chronic systemic therapy with immunosuppressive agents including corticosteroids.

7.2.12. Concurrent disease or condition that would make the patient inappropriate for trial participation or any serious medical disorder that would interfere with the patient's safety.

7.2.13. Known history of uncontrolled hypertension ($\geq 180/110$), unstable diabetes mellitus, dyspnea at rest, or chronic therapy with oxygen.

7.2.14. Dementia, altered mental status, or any psychiatric condition that would prevent the understanding or rendering of Informed Consent.

7.2.15. Treatment with an investigational agent in the 4 weeks before enrollment.

7.2.16. Active autoimmune disease or a documented history of autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Patients with vitiligo or resolved childhood asthma/atopy would be an exception to this rule.



Patients that require intermittent use of bronchodilators or local steroid injections would not be excluded from the trial. Patients with hypothyroidism stable on hormone replacement or Sjögren's syndrome will not be excluded from the trial.

- 7.2.17. Chemotherapy, radioactive therapy, and/or biological cancer therapy within 3 weeks prior to the first trial dose and has not recovered to CTCAE v.4.0 grade 1 or better from adverse events.
- 7.2.18. Pregnant or lactating women; lactation has to stop before enrollment.
- 7.2.19. The patient of childbearing potential who is unwilling to use **highly** effective contraception during treatment and up to **7 months** after stop of trial treatment. Acceptable **methods** are **intrauterine devices (without hormones), bilateral tubal occlusion, vasectomized partner** or total abstinence. Oral, injectable, or implant hormonal contraceptives are not allowed.
- 7.2.20. Unresolved or unstable, serious adverse events from prior administration of another investigational drug.
- 7.2.21. Active or uncontrolled infection CTCAE v.4.0 grade 2 or higher.
- 7.2.22. Live vaccines within 30 days prior to the first dose of trial therapy and during trial treatment.

7.3. **Inclusion criteria for enrollment**

The criteria from Section 7.1 apply for enrollment as well. The following criteria should be (re-)checked at the time of enrollment. The patient may only be included in the trial if ALL the following inclusion criteria are fulfilled:

- 7.3.1. Central lab confirmation on a metastatic biopsy (or biopsy from unresectable loco-regional disease) of
 - HER2-positivity (immunohistochemistry score 3+) or ERBB2-amplification (Ratio ERBB2/centromeres ≥ 2.0 or mean gene copy number ≥ 6),
 - Presence of PD-L1 expression assessed by IHC (**During the phase II portion of the trial a parallel, secondary cohort of 15 patients with PD-L1 negative disease will be enrolled, see section below**).

Note for the secondary cohort of 15 patients with PD-L1 negative disease:

Patients previously screened (under protocol version 1.1 or 1.2) with a negative PD-L1 result can be included once Amendment 1 has been approved, provided all eligibility criteria as specified in this protocol version are met, all tests and scans have been performed within the timelines specified, and biopsy is ≤ 1 year from enrollment. Patients must have demonstrated progression on current treatment. Center will need to register the patient again and get a new patient ID in the IBCSG Registration/Randomization System.



7.3.2. Patient agrees to submit tumor tissue for translational research:

- tissue biopsy from unresectable loco-regional or metastatic disease obtained ≤ 1 year prior to enrollment or new tissue material from a recently obtained surgical or diagnostic biopsy. For patients who have presented with stage 4 disease de novo, a biopsy taken from the presumed primary breast lesion is acceptable, (provided this was taken ≤ 1 year prior to enrollment).
- if available: FFPE tumor block from primary tumor.
- if available: pre-treatment fresh frozen tumor biopsy.
- if feasible: FFPE tumor block from post-treatment biopsy will be taken at time of disease progression or end of all treatment if ended prior to progression. This re-biopsy is strongly advised.
- if feasible: fresh frozen tumor biopsy from post-treatment biopsy will be taken at time of disease progression or end of all treatment if ended prior to progression.

7.3.3. Patient agrees to submit baseline (pre-treatment) blood and serial plasma for translational research, as detailed in Table 9.

7.3.4. For patient of childbearing potential, negative serum pregnancy test. Pregnancy test has to be repeated within 72h before treatment start.

7.3.5. All anti-cancer treatment including endocrine therapy, with the exception of trastuzumab must stop 3 weeks prior to first dose of trial treatment.

* ECOG Performance Status:

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

7.4. **Exclusion criteria for enrollment**

All criteria from Section 7.2 apply for enrollment as well. Excluded are patients who have received any of the treatments below:

7.4.1. Live vaccines within 30 days prior to the first dose of trial therapy and during trial treatment.

7.4.2. History of CNS metastases or spinal cord compression if they have not been clinically stable for at least 4 weeks before first dose of investigational product and require high-dose steroid treatment.

7.4.3. Treatment with an investigational agent in the 4 weeks before enrollment.



- 7.4.4. Patient has not recovered to CTCAE v.4.0 grade 1 or better from adverse events of prior therapy, **except alopecia grade 2**.
- 7.4.5. **Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), or positive for Hepatitis B (HBsAg reactive) or Hepatitis C (HCV RNA [qualitative]).**

8. Patient registration for screening and enrollment

This trial will use a web-based registration system. Specific details for registration are in the “*IBCSG Registration/Randomization Procedures Manual*” which is available on the IBCSG website (www.ibcsg.org).

Note: In the phase Ib and in phase II the trial may suspend the enrollment of additional patients. The IBCSG Registration/Randomization System will display a message indicating whether the trial currently can accept more patients. A slot reservation procedure **for enrollment** will be implemented; prior to **enrolling** a patient, the site should contact IBCSG to ascertain that there is an open slot for this patient. The Data Management Center will reserve the slot for the site. **Please refer to the “*IBCSG 45-13 PANACEA- Enrollment Slotting Procedures*” for more details.**

8.1. Registration for screening

- 8.1.1. Patient needs to give informed consent to trial participation and screening procedures and central determination of eligibility, prior to any screening intervention. If tumor material from a biopsy of a metastatic or unresectable loco-regional lesion (newly taken or obtained ≤ 1 year prior to enrollment) is available, then this material can be used for central determination. Otherwise, patient needs to consent to new biopsy.
- 8.1.2. Verify eligibility (see Section 7, including local determination of HER2-positivity). **In general**, screening procedures need to be done within 28 days before start of treatment (**see Section 14.1 for exceptions**).
- 8.1.3. Directly access the IBCSG Registration/Randomization System and provide the requested information as indicated on the Screening Form. The date the Informed Consent Form and the Biological Material Consent were signed by the patient and the date signed by the Investigator are both required to complete registration for screening.

The IBCSG Registration/Randomization System will provide the following information via e-mail:

- Patient ID (registration number)
- Date of registration



- 8.1.4. Submit the Screening (45-A1) electronic case report form (eCRF) via iDataFax. The patient binder of eCRFs will be available in iDataFax within 24 hours of successful registration.
- 8.1.5. Ship FFPE block from the metastatic tumor biopsy to the central laboratory for determination of HER2 status. The central laboratory will forward five 5 µm sections (FFPE) to the certified laboratory for PD-L1 status determination. **The determination of HER2 and PD-L1 status will take approximately 2 weeks.**

8.2. Prior to enrollment

All patients will have central review of the biopsy to assess HER2-positivity and PD-L1 status. For the primary objectives, only patients with central confirmation of HER2-positivity and presence of PD-L1 expression on metastatic biopsy (or biopsy from unresectable loco-regional disease) after registration for screening will be eligible to enroll. During the phase II portion of the trial, a secondary cohort of 15 patients with PD-L1 negative disease will be enrolled. Results from HER2 and PD-L1 testing will be communicated to the Investigator. If the patient meets the eligibility guidelines, the Trial Coordinator will open an enrollment slot for that Center. If the first submitted sample tests negatively, the Center has the option to submit a second sample.

Complete the following steps to enroll a patient.

- 8.2.1. Verify eligibility for enrollment (see Section 7). **If not done within 7 days before expected start of treatment, repeat hematology and chemistry lab.**
- 8.2.2. Directly access the IBCSG Registration/Randomization System, enter the Patient ID assigned at screening, and provide the requested information as indicated on the Confirmation of Enrollment (45-A2) Form.

8.3. Registration for Enrollment

Complete the following steps to enroll a patient on this trial.

- 8.3.1. Submit the Confirmation of Enrollment (45-A2) electronic case report form (eCRF) via iDataFax. The patient binder of eCRFs will be available in iDataFax within 24 hours of successful registration.
- 8.3.2. The site will receive an e-mail with information about the dose level of MK-3475 to use for the patient.

8.4. Registration Help Desk

The IBCSG Data Management Center (located at Frontier Science (FSTRF)) is responsible for developing and maintaining the IBCSG Registration/Randomization System. The Help Desk includes technical personnel and administrators of the registration programs at the Data Management Center in Amherst, NY, USA.



Business Hours: 24 hours. Closed five US holidays: New Year's Day, Memorial Day, Independence Day, Thanksgiving Day and Christmas Day. Refer to the DMC Holiday Calendar on the IBCSG website for specific dates.

FSTRF Randomization Help Desk
Frontier Science & Technology Research Foundation (FSTRF)
4033 Maple Rd, Amherst, NY 14226 USA
Phone: +1 716-834-0900 x7301 or 716-834-0900 x7200
Fax: +1 716-832-8437
Email: bc.helpdesk@fstrf.org

9. Trial drugs formulation and handling

MK-3475 is the Investigational **Medicinal** Product (IMP) used in this trial; IMP will be supplied.

Trastuzumab will also be supplied **in Europe**.

Complete details of the trial drug logistics, distribution, packaging, labeling and handling as well as accountability are described in a separate **Drug Supply Manual**.

9.1. MK-3475

MK-3475 (previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection.

9.1.1. Formulation

Refer to the current version of the MK-3475 Investigator's Brochure for pharmaceutical formulation information.

Clinical Supplies will be provided by Merck as follows:

Product Name & Potency	Dosage Form
MK-3475 50 mg/ vial	white to off-white lyophilized powder for infusion, it is reconstituted with sterile water for injection prior to use
The drug product is stored under refrigerated conditions (2°C - 8°C). The reconstituted lyophilized product is intended for i.v. administration.	

9.1.2. Packaging and labeling

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



9.1.3. Clinical Supplies Disclosure

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

9.1.4. Storage and handling

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. The Investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

9.2. **Trastuzumab**

9.2.1. Packaging and labeling

Commercial trastuzumab will be affixed with a clinical label in accordance with regulatory requirements.

Each vial contains 150 mg powder for concentrate for solution for infusion (i.v.) of trastuzumab. Each carton contains one vial.

9.2.2. Storage and handling

Store trastuzumab in a refrigerator (+2°C – +8°C). Instructions for proper handling and disposal of trastuzumab should be followed. All drugs will be stored as per the current version of the product's SPC (Summary of Product Characteristics) and the standard hospital procedures. Pharmacy will maintain temperature logs of all storage conditions and comply with hospital pharmacy standard operating procedures.

10. **Treatment**

10.1. **Trial treatments**

Trial treatment should start within one week after enrollment. Trial treatments will be administered in 3-week (21-day) cycles until progression, lack of tolerability, completion of 24 months of treatment with MK-3475, or until further protocol treatment is declined. Trastuzumab can be continued in case of MK-3475 cessation due to toxicity. Similarly, MK-3475 can be continued if trastuzumab has been ceased. In case of cardiac dysfunction both drugs must be stopped.

Treatment administration should comply with the protocol; compliance will be monitored by the Monitoring Team or Data Management Center. Complete details of dispensation and dosing are recorded on the eCRF.

10.2. **Phase Ib: dose finding and DLTs**

The primary objective of the phase Ib part of the trial is to determine the recommended phase II dose of the anti-PD-1 mAb, MK-3475, in combination with standard dose



trastuzumab. A patient in the phase Ib portion of the trial will be replaced if determination of DLT cannot be adequately assessed during the first cycle of therapy because of rapid disease progression, or if treatment and/or follow-up is stopped during the first cycle of therapy for reasons other than toxicity. Patients who do not receive trastuzumab in the first cycle of therapy, e.g., due to an infusion reaction to MK-3475, will also be replaced, but will not be counted as having a DLT. Replaced patients will be followed for safety.

10.2.1. Definition of dose-limiting toxicity

A dose-limiting toxicity (DLT) is defined as an adverse event or abnormal laboratory value assessed as suspected to be trial treatment related (possible, probable or definite) and unrelated to disease or disease progression that occurs within the first cycle (21 days) of treatment with MK-3475 and trastuzumab. Toxicities and lab values will be graded according to CTCAE v4.0. Dose-limiting toxicities will be defined as:

- any grade-3 or greater non-hematological adverse event lasting at least one week
- OR
- any grade-4 hematological toxicity,
- OR
- adverse event resulting in >14 days delay in starting cycle 2.

10.2.2. Dose-escalation rules

Dose escalation will occur using a standard ‘3+3’ dose escalation approach, beginning in dose level 1, with rules for escalation and de-escalation described in Table 1, below. The RP2D is defined as the highest dose level at which <33% (0 of three patients, or 0 or 1 of six patients) has experienced a DLT in cycle 1. Once dose escalation for MK-3475 reaches a dose of 10 mg/kg, no further escalation will occur.

Intra-patient dose escalation is not permitted. Patients enrolled in the phase Ib part of the trial should be treated on the assigned dose level as long as tolerable, for up to 24 months of MK-3475.

Table 1 Dose escalation rules

Number of patients with DLT at a given dose level	Dose Escalation Rule
0 out of 3	Proceed to the next dose level and enroll 3 patients
1 out of 3	Enroll and treat 3 additional patients at this dose level.
≥2 out of 3	Dose escalation will be stopped. The RP2D will be one dose below this dose level.



Number of patients with DLT at a given dose level	Dose Escalation Rule
1 out of 6	Proceed to the next dose level.
≥ 2 out of 6	Dose escalation will be stopped. The RP2D will be one dose below this dose level.
If $\geq 2/3$ or $\geq 2/6$ patients at dose level 1 experience DLTs, dose level -1 will be used. If dose level -1 proves too toxic, the trial will stop.	

10.2.3. Dose levels

All patients will be treated with the same dose of trastuzumab: 6 mg/kg i.v. (8 mg/kg i.v. in cycle 1, in case the last dose of trastuzumab was administered more than 3 months ago).

At enrollment, IBCSG will notify the Center about which dose level of MK-3475 to use.

Table 2 Body weight based dose levels

Dose Level	MK-3475	Trastuzumab*
-1	1 mg/kg i.v.	6 mg/kg i.v.
1	2 mg/kg i.v.	6 mg/kg i.v.
2	10 mg/kg i.v.	6 mg/kg i.v.

* 8 mg/kg i.v. in cycle 1 if last dose trastuzumab administered more than 3 months prior

Within-patient dose escalation for MK-3475 is not permitted.

10.3. Phase II dose of MK-3475

The phase II part of this trial will use MK- 3475 at a flat dose of 200 mg. The dose of MK-3475 used in the study may be adjusted based upon emerging data. IBCSG reserves the right to adjust the dose without amending the protocol. Investigators will be notified in writing before such a change takes place.

10.4. Treatment Administration

10.4.1. MK-3475 Administration

MK-3475 will be administered as a 30-minute i.v. infusion every 3 weeks (+/- 3 days). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Planning the time of trial drug infusion (e.g., time of the week for first administration; time of the day for each administration) should take trial visit procedures into consideration.



The pharmacists will prepare the MK-3475 medication for administration. The **Drug supply Manual** contains specific instructions for MK-3475 dose calculation, reconstitution, preparation of the infusion fluid, and administration. This document is available for reference by the pharmacist and trial personnel.

10.4.2. Trastuzumab administration

Trastuzumab will be administered at a dose of 6 mg/kg, every 3 weeks (+/- 3 days) (cycle duration: 21 days). If in cycle 1 a loading dose of trastuzumab of 8 mg/kg is administered, this has to be done over 90 minutes. In further cycles, trastuzumab will be administered over 30 minutes. The administration is by i.v. infusion according to local guidelines. A loading dose of 8 mg/kg i.v. trastuzumab in cycle 1 is needed if trastuzumab was stopped more than 3 months before start of treatment in this trial.

Trastuzumab should be given after MK-3475. In case of infusion reaction to MK-3475, delay the administration of trastuzumab and re-start MK-3475, within the same cycle, according to Table 5 in Section 10.6. If no second infusion reaction, then administer trastuzumab.

10.5. Dose modifications and delays for trastuzumab

Refer to standard of care guidance or current clinical practice for patients receiving trastuzumab treatment.

Efforts should be made so that the patient misses no more than one dose of three-weekly trastuzumab.

Table 3 Actions to be taken in case of trastuzumab related adverse events

Adverse Events graded according to NCI-CTCAE v.4.0	Action
Non-hematological, grade 1 or 2 (excluding cardiac)	Continue trastuzumab therapy.
Non-hematological, grade 3 or 4 (excluding cardiac), and adverse events resolved to grade ≤ 2 within a maximum of 5 weeks from last administration.	Hold trastuzumab therapy until recovery to grade ≤ 2 .
Non-hematological, grade 3 or 4 (excluding cardiac), and adverse events NOT resolved to grade ≤ 2 within a maximum of 5 weeks from last planned administration.	Discontinue trastuzumab therapy permanently.
Non-hematological, grade 3 or 4 (excluding cardiac), upon rechallenge with trastuzumab	Discontinue trastuzumab therapy permanently.



Adverse Events graded according to NCI-CTCAE v.4.0	Action
Cardiac* (asymptomatic drop in LVEF or symptomatic congestive heart failure)	Trastuzumab therapy to be held, continued, or resumed according to Figure 1. Trastuzumab therapy to be discontinued permanently in case of symptomatic CHF.
Cardiac (CTCAE: other cardiac toxicities not covered in Figure 1)	Actions must follow rules for non-hematological toxicities (above).
Hematological	Trastuzumab dose should not be held.

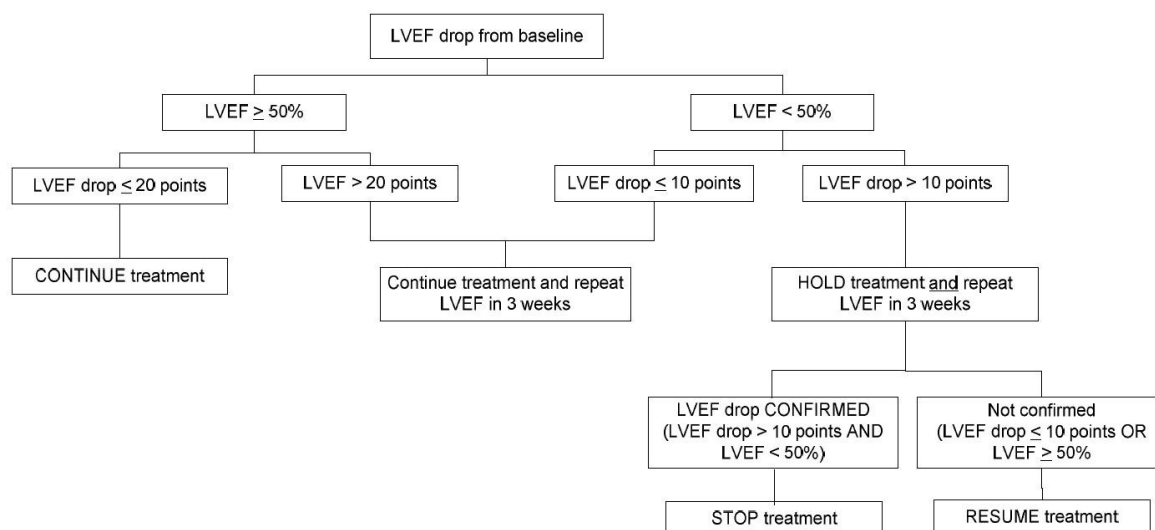
*Severity corresponding to NYHA functional classification:



Table 4 NYHA functional classification

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
I	Patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

Figure 1: Algorithm for continuation and discontinuation of trastuzumab based on interval LVEF assessments, for patients with NYHA class I or II congestive heart failure. LVEF drop > 10 points: Temporarily discontinue both drugs and repeat LVEF in 3 weeks. If LVEF drop > 10 points not confirmed, then resume treatment and administer MK-3475 at the same dose level.



10.6. Dose modifications and delays for MK-3475

Dose increase of MK-3475 will not be permitted. Dose reductions in phase II are not permitted.

10.6.1. Dose reductions in phase Ib

Only the three **body weight-based** dose levels in Table 2 in Section 10.2 may be used. If dose level -1 is not tolerated by the patient, MK-3475 has to be permanently discontinued.

10.6.2. Dose delays and supportive care for phase Ib and phase II

Adverse events (both non-serious and serious) associated with MK-3475 exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. MK-3475 must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 5 below.



Table 5 Treatment delay and supportive care guidelines for drug-related adverse events

Toxicity	Grade	Treatment delay/stop	Supportive care
Diarrhea/Colitis	Any grade		<p>Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).</p> <p>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 i.v. infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.</p>
	2	Restart treatment once toxicity resolves to Grade 0-1. If toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks, stop treatment.	Administer oral corticosteroids ¹ . 1–2 mg/kg/day prednisone or equivalent followed by a taper.
	3	Restart treatment once toxicity resolves to Grade 0-1. If toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks, stop treatment.	Treat with intravenous steroids followed by high dose oral steroids ¹ . 1–2 mg/kg/day prednisone or equivalent followed by a taper.
	4	Permanently discontinue.	Treat with intravenous steroids followed by high dose oral steroids ¹ . 1–2 mg/kg/day prednisone or equivalent followed by a taper.



Toxicity	Grade	Treatment delay/stop	Supportive care
AST, ALT, or increased Bilirubin	2	Restart treatment once toxicity resolves to Grade 0-1. Stop treatment if toxicity does not resolve within 12 weeks of last dose.	Monitor liver function tests more frequently until returned to baseline values (consider weekly). Treat with i.v. or oral corticosteroids ¹ . Initial dose of 0.5–1 mg/kg/day prednisone or equivalent followed by a taper.
	3-4	Permanently discontinue Exception: patients with liver metastasis who begin treatment with Grade 2 AST or ALT can resume treatment, if AST or ALT increases by less than 50% relative to baseline and for less than 1 week.	Treat with intravenous corticosteroids for 24 to 48 hours ¹ . 1–2 mg/kg/day prednisone or equivalent followed by a taper.
Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)	T1DM or 3-4	Hold MK-3475 for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure. Resume MK-3475 when patients are clinically and metabolically stable.	Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria. Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.



Toxicity	Grade	Treatment delay/stop	Supportive care
Hypophysitis	2	Restart treatment once toxicity resolves to Grade 0-1. Therapy with MK-3475 can be continued while endocrine replacement therapy is instituted. If toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks, stop treatment.	Treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
	3-4	Restart treatment once toxicity resolves to Grade 0-1. Therapy with MK-3475 can be continued while endocrine replacement therapy is instituted. If toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks, stop treatment.	Treat with an initial dose of i.v. corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
Hyperthyroidism	2		Non-selective beta-blockers (e.g., propranolol) are suggested as initial therapy.
	3	Restart treatment once Toxicity resolves to Grade 0-1. Stop treatment if toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.	Treat with an initial dose of i.v. corticosteroid followed by oral corticosteroids ¹ Replacement of appropriate hormones may be required as the steroid dose is tapered.
	4	Permanently discontinue.	Treat with an initial dose of i.v. corticosteroid followed by oral corticosteroids ¹ Replacement of appropriate hormones may be required as the steroid dose is tapered.



Toxicity	Grade	Treatment delay/stop	Supportive care
Hypothyroidism	2-4	Therapy with MK-3475 can be continued while thyroid replacement therapy is instituted.	Thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
Infusion Reaction			Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Table 6 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of MK-3475.
	2	Restart treatment once toxicity resolves to Grade 0-1. Permanently discontinue if toxicity develops despite adequate premedication.	If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to Table 6 – Infusion Reaction Treatment Guidelines for further management details.
	3-4	Permanently discontinue	
Pneumonitis	Any grade		Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
	2	Restart treatment once toxicity resolves to Grade 0-1. Discontinue treatment if toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. Permanently discontinue if event recurs.	Treat with systemic corticosteroids ¹ . Initial dose of 1–2 mg/kg/day prednisone or equivalent followed by a taper.



Toxicity	Grade	Treatment delay/stop	Supportive care
	3-4	Permanently discontinue treatment.	Immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
Renal Failure or Nephritis	2	Restart treatment once toxicity resolves to Grade 0-1. Stop treatment if toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.	Treat with corticosteroids ¹ . Initial dose of 1–2 mg/kg/day prednisone or equivalent followed by a taper.
	3-4	Permanently discontinue	Treat with systemic corticosteroids ¹ . Initial dose of 1–2 mg/kg/day prednisone or equivalent followed by a taper.
All Other Drug-Related Toxicity	2	Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.	
	3 or Severe	Restart treatment once toxicity resolves to Grade 0-1. Stop treatment if toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.	
	4	Permanently discontinue	

1) When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care.

Note: Permanently discontinue for any Grade 3 (Grade 2 for pneumonitis) drug-related AE that recurs, or any life-threatening event.



In case toxicity does not resolve or improve to \leq Grade 1 within 12 weeks after last administration of MK-3475, MK-3475 **should be discontinued**. In **exceptional cases, continuation may** be considered after discussion with IBCSG (ibcsg45_panacea@fstrf.org and medical.affairs@ibcsg.org). With Investigator and IBCSG agreement, patients still at Grade 2 may continue in the trial only if asymptomatic and controlled.

Two dosing delays due to the same toxicity will be permitted. In the event of a third occurrence of the same toxicity, which would require dosing delay, MK-3475 will be discontinued permanently.

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

Table 6 shows treatment guidelines for patients who experience an infusion reaction associated with administration of MK-3475.



Table 6 Infusion reaction treatment guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
<p><u>Grade 1</u> Mild reaction; infusion interruption not indicated; intervention not indicated</p>	<p>Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the Investigator.</p>	<p>None.</p>
<p><u>Grade 2</u> Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, i.v. fluids); prophylactic medications indicated for <=24 hrs</p>	<p>Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: i.v. fluids Antihistamines NSAIDS Acetaminophen Narcotics</p> <p>Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the Investigator.</p> <p>If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the patient should be premedicated for the next scheduled dose.</p> <p>Patients who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.</p>	<p>Patient may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with:</p> <p>Diphenhydramine 50 mg p.o. (or equivalent dose of antihistamine).</p> <p>Acetaminophen 500-1000 mg p.o. (or equivalent dose of antipyretic).</p>
<p><u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated</p>	<p>Stop Infusion. Additional appropriate medical therapy may include but is not limited to: i.v. fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine</p> <p>Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the Investigator.</p> <p>Hospitalization may be indicated.</p> <p>Patient is permanently discontinued from further trial treatment administration.</p>	<p>No subsequent dosing.</p>
<p>Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov</p>		



10.7. Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The Investigator should discuss any questions regarding this with IBCSG. The final decision on any supportive therapy or vaccination rests with the Investigator and/or the **patient's** primary physician. However, the decision to continue the **patient** on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, IBCSG, and the **patient**.

10.7.1. Acceptable Concomitant Medications

All treatments that the Investigator considers necessary for a **patient's** welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the eCRF including all prescription, over-the-counter (OTC), herbal supplements, and i.v. medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date must also be included on the CRF.

All concomitant medications received within 28 days before **start of trial treatment** and up to 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 12.4.

10.7.2. Prohibited Concomitant Medications

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

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Endocrine treatment (SERMs, aromatase inhibitors, GnRH analogs)
- Chemotherapy not specified in this protocol
- Investigational agents other than MK-3475
- Radiation therapy

Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with IBCSG (medical.affairs@ibcsg.org and ibcsg45_panacea@fstrf.org).

- Live vaccines within 30 days prior to the first dose of trial treatment and while under trial treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.



- Glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology. The use of physiologic doses of corticosteroids (prednisone 10 mg orally daily or equivalent) is at the discretion of the Investigator.

The exclusion criteria in Section 7.2 describe other medications, which are prohibited in this trial. There are no prohibited therapies during the post-treatment follow-up phase.

10.8. Diet/Activity/Other Considerations

10.8.1. Diet

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

10.8.2. Contraception

MK-3475 may have adverse effects on a fetus in utero. Non-pregnant, non-breast-feeding women of childbearing potential may be enrolled if they are willing to use effective contraception. Allowed are: **intrauterine devices (without hormones), bilateral tubal occlusion, vasectomized partner** or total abstinence. Oral, injectable, or implant hormonal contraceptives are not allowed. Patients should start using birth control from the start of trial treatment throughout the trial period up to **7 months** after the last dose of any trial treatment (MK-3475 and/or trastuzumab).

Patients should be informed that taking the trial medication may involve unknown risks to the fetus if pregnancy were to occur during the trial. In order to participate in the trial they must adhere to the contraception requirement (described above) for the duration of the trial **treatment and up to 7 months after the last dose of any trial treatment**. If there is any doubt whether a patient will reliably comply with the requirements for contraception, that patient should not be entered into the trial.

10.8.3. Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with MK-3475, trial treatment will be stopped immediately for the patient and the event reported immediately, see Section 12.5. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to IBCSG 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 trial 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 IBCSG.

10.8.4. Use in Nursing Women

It is unknown whether MK-3475 is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breast-feeding are not eligible for enrollment.



10.9. Stop of treatment

Patients will receive combination trastuzumab and anti-PD-1 mAb until progression, lack of tolerability, completion of 24 months of treatment with MK-3475, or until further protocol treatment is declined **by the patient. 24 months of MK-3475 is calculated from the date of first dose.** Trastuzumab can be continued until progression in case of MK-3475 cessation due to toxicity. Similarly, MK-3475 can be continued until progression if trastuzumab has been ceased, except in the case of cardiac dysfunction where both drugs must be stopped.

Asymptomatic patients may receive trial treatment for a maximum of 24 months.

10.9.1. Criteria to continue treatment in case of progression

The first radiological assessment is at week 12, unless progressive disease is suspected earlier. Imaging should then be at weeks 18, 24 and every 12 weeks after this, or earlier if clinically indicated.

A patient with *unconfirmed* progression of disease should continue trial treatment until progression of disease according to RECIST 1.1 is confirmed by radiographic imaging obtained at 4-6 weeks later.

Patients with confirmed progression of disease may still benefit from the continuation of trial treatment. This includes patients who develop new symptomatic brain lesions without extracranial progression. Continuation of trial treatment for such patients must be discussed with IBCSG (please contact ibcsg45_panacea@fstrf.org and medical.affairs@ibcsg.org).

In both cases (confirmed and unconfirmed progression), patients have to meet the following criteria to continue treatment:

- Absence of signs and symptoms (including significant laboratory values) indicating **worsening of** disease.
- 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.

10.9.2. Criteria to stop trial treatment

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. Asymptomatic patients may receive trial treatment for a maximum of 24 months.

The treatment of the individual patient will be discontinued in case of:

- Confirmed disease progression as defined in Section 13.10, **except for patients who may benefit from treatment continuation, as described at the end of Section 10.9.1.**
- Cardiac dysfunction.
- Unacceptable adverse event(s).
- Intercurrent illness that prevents further administration of trial treatment.



- Need for more than 2 dose delays due to the same toxicity as per dose modification guidelines described in Section 10.5.
- Patient demonstrates an inability or unwillingness to comply with the treatment regimen and/or trial requirements.
- Patient has confirmed positive serum pregnancy test.
- Patient declines further trial treatment.
- General or specific changes in the patient's condition which render her unacceptable for further trial treatment in the opinion of the treating Investigator.
- Patient withdraws consent to continue trial treatment.

Patients who have a confirmed complete response (CR) by two scans ≥ 4 weeks apart and who have been on MK-3475 treatment for at least 6 months may discontinue MK-3475 treatment at the discretion of the Investigator after receiving at least two doses beyond the initial determination of CR, whereas trastuzumab can continue. MK-3475 and trastuzumab may be resumed upon disease recurrence in these patients, provided they fulfill the original clinical and laboratory value eligibility criteria (see Section 7). Patients will resume therapy at the same dose and schedule used at the time of initial discontinuation. If a patient with a CR has discontinued treatment but then resumes treatment upon disease recurrence, the procedures will be followed as described in Section 14.

For scheduling reasons or patient's personal reason unrelated to toxicity, patients who have been on MK-3475 trial therapy for at least 24 weeks may have a treatment-free interval of up to 12 weeks if considered in a patient's best interest by the Investigator. Any longer interval requires discussion with and approval by IBCSG on an individual basis.

11. Safety

11.1. Adverse effects of MK-3475

As of 30 June 2015, 9400 patients had been treated with MK 3475 at several dose schedules. The safety of MK-3475 has been evaluated in 1012 patients across three doses (2 mg/kg every 3 weeks or 10 mg/kg every 2 or 3 weeks) in clinical studies. In this patient population, the most common adverse reactions (>10%) with MK-3475 were diarrhea (15%), nausea (12%), pruritus (25%), rash (25%), arthralgia (13%) and fatigue (33%). The majority of adverse reactions reported were of Grade 1 or 2 severity. The most serious adverse reactions were immune-related adverse reactions and severe infusion-related reactions.

As of 14 August 2015, data of 409 patients treated with 200 mg flat dose were reviewed. A review of these adverse events demonstrated them to be consistent with the known adverse event profile for MK-3475; no new significant findings were evident.



11.2. Immune-related adverse effects of MK-3475

An irAE is defined as a clinically significant adverse event of any organ that is associated with trial drug exposure, is of unknown etiology, and is consistent with an immune-related mechanism.

Immune-mediated adverse reactions are presented based on 2117 patients with melanoma and with NSCLC. The safety profile was generally similar for patients with melanoma and NSCLC. The most commonly reported immune-related adverse events across the dose-schedules are hypothyroidism (7.8%), hyperthyroidism (2.9%), pneumonitis (2.4%), and colitis (1.7 %).

For the occurrence and management of immune-related adverse effects, please refer to Section 10.6.2 in conjunction with the latest version of the MK-3475 Investigator's Brochure. See Section 12.3 for an overview of ECIs.

11.3. Drug interactions with MK-3475

No conventional drug-drug interactions (DDI) that could affect pharmacokinetics of either MK-3475 or trastuzumab are to be expected (and have not been described). Therefore, no dedicated DDI study is required.

11.4. Adverse effects of trastuzumab

See the updated IB or SPC for adverse effects of trastuzumab.

11.5. Safety Assessment in stage 1 of phase II

Data from the first-in-human phase I trial of MK-3475 in patients with metastatic melanoma and other solid tumors were presented at the American Society of Clinical Oncology (ASCO) 2012 annual meeting. In the dose-escalation phase of the melanoma trial, MK-3475 appeared to be well-tolerated at 1, 3, and 10 mg/kg administered every 2 weeks, with no immunogenicity or dose-limiting toxicities reported. However, there is no experience of combining MK-3475 with trastuzumab. Thus, as an early assessment of safety, data from the initial 17 patients in the phase II primary cohort with PD-L1 expressing disease receiving the combination of MK-3475 and trastuzumab will be evaluated for safety in the first 4 cycles of therapy. This evaluation will coincide with the first assessment of response for the two-stage design. Recruitment into the trial will be interrupted after enrollment of the 17th patient with PD-L1 expressing disease if two objective responses have not yet been observed in that cohort. The safety assessment will also include data from patients in the secondary cohort with PD-L1 negative disease who are enrolled prior to the 17th patient with PD-L1 expressing disease. The incidence of the following cardiac events will be analyzed:

- cardiac death
- heart failure manifested by dyspnea with normal activity or at rest
- decline in LVEF of >10 percentage points from baseline and to a value <50 percent.



The report will be submitted within one month to the DSMC. **From the perspective of safety**, the trial will **continue (or resume)** enrollment should the incidence of such events not be greater than what would be expected for trastuzumab alone (5%) (see also Section 17.4).

Rationale: HER2 signaling is important for embryonic cardiac development, myocyte survival as well as protection from potential cardiotoxins. Trastuzumab-related cardiac toxicity is thought not to be dose-dependent and related to signaling inhibition (HER2 blockade) rather than myocyte destruction. Endomyocardial biopsies from patients with trastuzumab-related cardiac dysfunction do not show typical anthracycline-related damage. Interestingly however, lapatinib rarely causes cardiac dysfunction. Certain risk factors are associated with a higher risk of developing trastuzumab-related cardiotoxicity. These include previous or concurrent anthracycline use and age greater than 50. Concurrent treatment with trastuzumab and adjuvant radiation therapy does not seem to be associated with increased risk. Other risk factors are preexisting cardiac dysfunction (i.e., decreased left ventricular ejection fraction), older age, obesity, and hypertension, while diabetes, valvular heart disease, and coronary artery disease seem not to significantly increase risk. Trastuzumab-related cardiotoxicity is largely reversible in the majority of cases, and treatment can often be continued after resolution of cardiac abnormalities. Cardiac cells are thought to be stunned rather than permanently damaged.

In case of cardiac toxicity, the algorithm in Section 10.5 should be followed. Both MK-3475 and trastuzumab should be temporarily discontinued. Both can be re-introduced at the dose indicated in Section 10.5 after resolution as per cardiac algorithm.

All patients will undergo cardiac evaluation at 12 weeks with an assessment of LVEF, ECG, Troponin I and BNP. Antibodies against Troponin I are thought to be the pathogenesis of some causes of dilated cardiac myopathy (unrelated to trastuzumab). Following **this** first evaluation (and provided the **patients in the safety assessment** show no concerning safety signal) all other patients will undergo cardiac evaluations every 12 weeks [54].

12. Adverse event and serious adverse event reporting

12.1. Adverse event reporting

The main criterion for tolerability is the occurrence of toxicities and adverse events. The severity and causality will be classified according to the NCI CTCAE Version 4. The CTCAE is available for downloading on the internet at <http://evs.nci.nih.gov/ftp1/CTCAE/About.html>. An interactive version can be found at <https://safetyprofiler-ctep.nci.nih.gov/>.

An adverse event is defined as any untoward medical occurrence that occurs from the **date of signature of informed consent** until 30 days after all treatment discontinuation, regardless of whether it is considered related to a medication.

Any grade of any observed adverse event should be reported on the AE form. Symptoms of the targeted cancer (if applicable) should not be reported as adverse events.



An overdose, accidental or intentional, whether or not it is associated with an AE, of an investigational product should be reported as an SAE. **For the definition of overdose, see section 12.3.**

12.1.1. Severity / intensity

The adverse event severity grade provides a qualitative assessment of the extent or intensity of an adverse event, as determined by the Investigator or as reported by the patient. The severity grade does not reflect the clinical seriousness of the event, only the degree or extent of the affliction or occurrence (e.g., severe nausea, mild seizure), and does not reflect the relationship to trial drug.

Severity grade for other adverse events not covered in the toxicity grading scale:

Grade 1 = Mild – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required

Grade 2 = Moderate – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required

Grade 3 = Severe – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible

Grade 4 = Life threatening – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

Grade 5 = Death – the event results in death

The term “severe” is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is *not* the same as “serious” which is based on patient/event *outcome* or *action* criteria associated with events that pose a threat to a patient’s life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

12.1.2. Causality

The Investigator must determine the relationship between the administration of trial drug(s) and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below:

Not suspected	The temporal relationship of the adverse event to trial drug(s) administration makes a causal relationship unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.
Suspected	The temporal relationship of the adverse event to trial drug(s) administration makes a causal relationship possible, and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.



12.1.3. Duration

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event.

12.1.4. Action taken

The Investigator will report the action taken with trial drug(s) as a result of an AE or SAE, as applicable (e.g., discontinuation of trial drug(s)) and in case of an SAE report if concomitant and/or additional treatments were given for the event.

12.2. Serious adverse event (SAE) reporting

12.2.1. Definition

An SAE is defined, in general, as any undesirable medical occurrence/adverse drug experience that occurs during treatment or within 90 days after stopping all trial treatment that, at any dose, results in any of the following:

- fatal (any cause)
- life-threatening
- requires or prolongs inpatient hospitalization
- persistent or significant disability/incapacity
- congenital anomaly or birth defect (including neonatal deaths)
- secondary (non-breast) malignancy
- constitutes an important medical event
- event of clinical interest (see Section 12.3)

Important medical events are defined as those occurrences that may not be immediately life-threatening or result in death, hospitalization, or disability, but may jeopardize the patient or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

After completion of trial treatments, report all SAEs beyond 90 days that are considered at least possibly related to previous trial treatment. Cases of second (non-breast) malignancies and congenital abnormalities are to be regarded as SAEs, regardless of whether they occur during or after trial treatment. These events should be reported during the whole trial duration on the Serious Adverse Event eCRFs (SAE/**ECI-A** and SAE/**ECI-B**).

A suspected unexpected serious adverse reaction (SUSAR) is an adverse event that is serious, related to the investigational drug and not listed as a known toxicity of the investigational drug in the Investigator's brochure.

Note: Progression of the cancer under study must be reported as a serious adverse event if it has resulted in hospitalization or death.



12.2.2. Exceptions to the definition

Hospitalizations occurring under the following circumstances are not considered to be serious adverse events:

- elective surgery
- occur on an outpatient basis and do not result in admission (hospitalization <24h)
- are part of the normal treatment or monitoring of the studied treatment

12.3. Definition of Events of Clinical Interest

Selected non-serious and serious adverse events **described below** are also known as Events of Clinical Interest (ECI) and must be recorded as such on the SAE forms and reported within 24 hours to IBCSG. IBCSG will inform Merck Global Safety within one business day.

Overdose

An overdose is defined as ≥ 1000 mg (5 times the dose) of MK-3475 and as any dose $\geq 20\%$ over the prescribed dose for trastuzumab. No specific information is available on the treatment of overdose of MK-3475. In the event of overdose, MK-3475 should be discontinued and the patient should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated. If an adverse event(s) is associated with (“results from”) the overdose, then the adverse event(s) has to be reported as a serious adverse event, even if no other seriousness criteria are met. If a dose meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious ECI, using the terminology “accidental or intentional overdose without adverse effect.” All reports of overdose with and without an adverse event must be reported by the Investigator within 24 hours to IBCSG.

Drug-induced liver injury (DILI):

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, is considered drug-induced liver injury (DILI).

12.4. Reporting SAEs and ECIs

Any Serious Adverse Event and any Event of Clinical Interest occurring in a patient after providing Informed Consent must be reported, including death due to any cause other than progression of breast cancer, which occurs within 90 days following cessation of treatment or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the investigational product. Information about all such events will be collected and recorded on the IBCSG Serious Adverse Event eCRFs (SAE/ECI–A and SAE/ECI–B).



To ensure patient safety, the IBCSG must be informed of each SAE and each ECI using the procedures described below:

- The Investigator/MD responsible for the patient must complete a Serious Adverse Event (**SAE/ECI-A**) eCRF in English within 24 hours of awareness via iDataFax. A copy is automatically forwarded to the IBCSG Safety Office for medical review.
- Queries may be issued by the IBCSG Safety Office; a timely response by the Investigator to all SAE-related queries is crucial.
- Follow-up information should be completed, via iDataFax, on the Serious Adverse Event (SAE-B) eCRF as early as possible but within 15 days of the initial report, even if the event reported in the **SAE/ECI-A** eCRF is not yet resolved. If the event is not resolved within 15 days, revise the original Serious Adverse Event (SAE-B) eCRF in iDataFax to report the final resolution.
- All SAEs or ECIs that have not resolved upon discontinuation of the patient's participation in the trial must be followed until recovered, recovered with sequelae, not recovered (death due to another cause) or death (due to the SAE).
- If a non-serious adverse event becomes serious, this and other relevant follow-up information must also be provided within 24 hours.
- Photocopies of all examinations carried out with the dates on which they were performed should be sent by fax or DFSend into the DataFax system. Care should be taken to ensure that the patient's identity is protected and the patient's Registration ID Number is properly included on ALL pages of any reports. For laboratory results, include the laboratory normal ranges. Please also note on each page that the information is "SAE related" so it can be properly categorized in iDF.
- In the event the eCRF system is not working, the SAE Forms can be found in the trial site file or downloaded from the IBCSG trial webpage and sent via fax or DFSend into the DataFax system.

If an SAE/ECI (**SAE/ECI-A** and **SAE/ECI-B** forms) was submitted by fax or DFSend, the original forms and the fax confirmation sheet(s) must be kept at the Participating Center.

The IBCSG will inform Merck Global Safety and other appropriate persons about all SAEs and ECIs within 24 hours of receipt at the IBCSG.

The IBCSG will record the SAE/ECI and prepare a monthly SAE/ECI report. Principal Investigators will receive the summary report on a monthly basis, and these reports can be found on the IBCSG web site (www.ibcsg.org).

12.5. Pregnancy

Pregnancies and suspected pregnancies of a patient occurring during trial treatment, or within **7 months** of the last dose of trial drug(s), are considered immediately reportable events. Trial drug(s) are to be discontinued immediately and the patient instructed to return any unused portion of the trial drug(s) to the Investigator. The pregnancy, suspected



pregnancy, or positive pregnancy test must be reported within 24 hours of the Investigator's knowledge using the Pregnancy Form (Form 45-PREG) to the IBCSG who will inform MSD immediately.

The patient should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the patient until completion of the pregnancy, and must notify IBCSG immediately about the outcome of the pregnancy by completing the corresponding section on the pregnancy form (45-PREG).

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to trial drug(s) should also be reported within 24 hours of the Investigator's knowledge of the event using the SAE Forms.

12.6. Reference Safety Information

The Pembrolizumab/MK-3475 Investigator's Brochure Edition 10 Section 7 serves as reference document for the determination of the expectedness of the serious adverse events of MK-3475.

The currently EU approved Summary of Product Characteristics (SPC) of trastuzumab serves as reference safety information to assess the expectedness of trastuzumab related serious adverse events.

13. Disease assessment, response and progression (RECIST 1.1)

13.1. Introduction

All enrolled patients will be assessed for disease response and progression according to the revised Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) (Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-2247, 2009). In this trial, patients must have measurable disease (see definitions below). Patients will be re-evaluated after 4 cycles (12 weeks), 6 cycles (18 weeks), 8 cycles (24 weeks) and then every 12 weeks (every 4 cycles) until confirmed progression.

Objective response (primary endpoint) will be assessed using RECIST 1.1 criteria.

13.2. Methods of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during treatment and follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

CT scan is the best currently available and reproducible method to measure lesions selected for response assessment. CT scan should generally be performed using a ≤ 5 mm contiguous reconstruction algorithm. MRI is acceptable for certain situations (e.g., body scans).



Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules) and ≥ 10 mm. In the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT scan is preferable.

Ultrasound is not useful in assessment of lesion size and is not accepted as a method of assessment.

FDG-PET (fludeoxyglucose positron emission tomography) is not foreseen for regular response assessments. It may, however, be used to detect or confirm the appearance of new lesions. Attenuation correction CT scans performed as part of a PET/CT scan frequently show lower resolution; therefore, dedicated CT scans are preferred. However, if the site can demonstrate that the CT scan performed as part of a PET/CT is of the same diagnostic quality as a diagnostic CT scan (with i.v. and oral contrast), then the CT scan portion of the PET/CT can be used for RECIST measurements.

13.3. Measurability of tumor at baseline

13.3.1. Measurable disease

Measurable disease is defined as the presence of at least one measurable lesion.

Measurable lesions:

- **Tumor lesions** must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
 - 10 mm by CT scan (CT scan slice thickness no greater than 5mm).
 - 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
 - 20 mm by chest X-ray.

Reminder: A lesion in a previously irradiated area is not eligible for measurable disease.

- **Malignant lymph nodes:** to be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan, assuming the slice thickness is ≤ 5 mm. At baseline and in follow-up, only the short axis will be measured.

13.3.2. Non-measurable disease

Non-measurable disease is defined as lesions or sites of disease that cannot be measured.

Non-measurable lesions/sites of disease and special considerations:

- Small non-nodal lesions (longest diameter < 10 mm in CT scan).
- Small lymph nodes (short axis ≥ 10 and < 15 mm). Lymph nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed as measurable or non-measurable disease.



- Bone lesions. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.
- Leptomeningeal disease
- Ascites
- Pleural or pericardial effusion
- Inflammatory breast disease
- Lymphangitic involvement of skin or lung
- Cystic lesions. Cystic lesions thought to represent cystic metastases may be considered as measurable lesions. However, if non-cystic lesions are present, these are preferred as target lesions.
- Tumor lesions situated in a previously irradiated area, or subjected to other loco-regional therapy. Such lesions may be considered measurable if there has been demonstrated progression in the lesion.
- Abdominal masses/abdominal organomegaly identified by physical exam that are not measurable by reproducible imaging techniques.

13.4. Selection of target lesions

Target lesions should be identified, measured and recorded at baseline (Form 45-TEV-B). At baseline, there can be up to a maximum of 5 lesions representative of all involved organs, and up to 2 per organ. Target lesions should be selected on the basis of their size and their suitability for accurate repetitive measurements. A sum of diameters for all target lesions will be calculated and reported as the baseline sum of diameters. Lymph nodes selected as target lesions should always have the short axis recorded. All other lesions should always have their longest diameters recorded. The sum of diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

13.5. Selection of non-target lesions

Non-target lesions should be identified. All other lesions (or sites of disease) not identified as target lesions should also be recorded as non-target lesions at baseline.

For non-target lesions, measurements are not required, but the presence or absence of each should be noted throughout follow-up (Form 45-TEV). It is possible to record multiple non-target lesions as a single item on the eCRF (e.g., "multiple liver metastases").



13.6. Evaluation of target lesions (measurable disease)

All target lesions will be measured at each tumor assessment, and the sum of their diameters will be compared to previous assessments in order to assign the response status as specified below.

- **Complete Response (CR):** Disappearance of all target lesions. Lymph nodes selected as target lesions must each have reduction in the short axis to <10 mm in order for the response to be considered complete. In this case, the sum of diameters may be >0.
- **Partial Response (PR):** At least a 30% decrease in the sum of diameters of target lesions taking as reference the baseline sum of diameters.
- **Progression (PD):** At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum recorded on trial. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions (see Section 13.8) denotes disease progression.
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest sum of diameters recorded on trial.

Note: All target lesions, including lymph nodes, should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). If the radiologist does not feel comfortable assigning an exact measure and reports a lesion as "too small to measure", a default value of 5 mm should be recorded. If a target lesion is thought likely to have disappeared, use "0 mm."

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD.

13.7. Evaluation of non-target lesions

- **Complete Response (CR):** Disappearance of all non-target lesions; lymph nodes selected as non-target lesions must be non-pathological in size (<10 mm).
- **Non-CR/non-PD:** Persistence of one or more non-target lesions (non-CR).
- **Progression (PD):** Unequivocal progression of existing non-target lesions. Unequivocal means: comparable in magnitude to the increase that would be required to declare PD for measurable disease or an overall substantial increase in tumor burden that merits treatment discontinuation.



When no imaging is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesions are evaluated at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD.

13.8. Determination of new lesions

The appearance of any new malignant lesions denotes disease progression. The finding of a new lesion should be unequivocal (i.e., not attributable to differences in scanning technique or findings thought to represent something other than tumor). If a new lesion is equivocal, (e.g., because of its small size) the patient will stay on treatment (if the decision on PD is based on this lesion only).

Lesions or sites of disease found in a new location not included in the baseline scan (e.g., brain metastases) are considered new lesions.

Note: The "re-appearance" of a previously "disappeared" target or non-target lesion does not in itself necessarily qualify as PD; this is the case only if the overall evaluation meets the PD criteria, or if the patient was previously in CR.

13.9. Additional considerations

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

13.10. Confirmation

In this trial, a partial response (PR), complete response (CR) or disease progression (PD) needs to be confirmed by repeat clinical and radiological assessment 4 to 6 weeks after the first assessment.

In each situation, it is the date of the first radiological assessment that is declared as the date of response or of progression.

13.11. Determination of time point response

Based on the responses of target lesions, non-target lesions, and the presence or absence of new lesions, the overall response will be determined at each tumor evaluation time point, according to Table 7 below.

Table 7 Measurable disease - overall response

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR



CR	Non-CR / non-PD*	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

*Non-CR/non-PD should be used rather than SD for categorizing non-target lesions.

13.12. Determination of best overall response

Objective response (confirmed CR or PR as best overall response) is the primary endpoint of the phase II portion of the trial. Best overall response will be determined centrally by an Endpoint Review Committee (see Section 19.4) based on tumor assessment eCRFs.

13.13. Storage of imaging

CT/MRI images must be stored locally in electronic format for potential central review, see separate instructions on the IBCSG website.

14. Clinical and laboratory evaluations

14.1. Screening

14.1.1. Obtain informed consent for screening evaluations and trial participation.

14.1.2. Cardiac evaluation: ECG, echocardiography or MUGA scan has to be performed within 3 months before treatment start.

Results of the screening examinations should be available in the patient file at the time of registration of a new patient.

The following examinations should be done within a maximum of 28 days before start of treatment. If examinations were done prior to 28 days before start of treatment, they have to be repeated before treatment start. For examinations marked below with an * the results of a test within the timeline of the 28 days before treatment start do not need to be present before sending the tumor material to the central office. Eligibility for screening can be judged on tests performed earlier but the tests will have to be repeated before treatment start. In cases where central confirmation of HER2+ and PD-L1 expression status takes more than 10 working days the treatment start can be within 35 days of the examinations.

14.1.3. Clinical and radiological tumor assessments by CT scan or MRI.

14.1.4. Bone scan if clinically indicated.

14.1.5. * Physical examination including vital signs, ECOG Performance Status, body weight.

14.1.6. * Hematology: Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count). To be repeated if not done within 7 days



prior to first dose of trial treatment.

- 14.1.7. * Biochemistry: serum potassium, sodium, calcium, serum amylase, lipase and LDH;

Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;
kidney function tests: urea, creatinine and uric acid.

Note: Biochemistry, liver and kidney function labs needs to be repeated if not done within 7 days prior to first dose of trial treatment.

- 14.1.8. * For patients of childbearing potential: Serum pregnancy test; the test has to be repeated before treatment start, if treatment does not start within 72 hours of the previous test.

- 14.1.9. Medical history including details of malignancy: date of diagnosis, primary tumor type characteristics (histology, grade).

- 14.1.10. * Baseline symptoms (record on baseline adverse events form) and concomitant medication.

- 14.1.11. * CA 15-3

The following examinations should be done within a maximum of 42 days before start of treatment. If examinations were done prior to 42 days before start of treatment, they have to be repeated:

- 14.1.12. T3, T4, TSH

- 14.1.13. Coagulation profile: according to local standards

- 14.1.14. Hepatitis B and C test,

- 14.1.15. * HIV test (needed for enrollment, not for screening)

- 14.1.16. Urinalysis: proteins, glucose and blood using a dipstick.

14.2. Central confirmation of HER2+ and PD-L1 status

The HER2 and PD-L1 status need to be evaluated centrally before enrollment to determine the eligibility of the patient.

- 14.2.1. Ship FFPE block of unresectable loco-regional or metastatic biopsy to central laboratory. Shipment from the participating center to the IBCSG Central Pathology Office may take between 2-5 business days.

Note: Do not submit for central review if the metastatic or unresectable loco-regional biopsy tested HER2-negative locally.

- 14.2.2. Central laboratory will evaluate HER2. In case HER2-positivity is confirmed, the central laboratory will forward tissue to the certified lab for determination of PD-L1 status.

- 14.2.3. Results of HER2 and PD-L1 assessments will be communicated to the site and are



expected to be available within **10 business days from the time the FFPE block arrives at the IBCSG Central Pathology Office, in Milan, Italy.**

14.3. **Biological samples before, during and after treatment**

Please consult Section 15.1 for a complete schedule of biological samples (FFPE and fresh frozen tumor tissue, whole blood, plasma) to be taken at baseline, during and after treatment. The “*Blood Sample Logistics Manual*” gives details on collection, storage and shipment.

14.4. **Day 1 of every treatment cycle**

Investigations marked with * need to be repeated prior to start of treatment if not done within 3 days prior to this day.

14.4.1. Physical examination including vital signs, ECOG Performance Status, and body weight.

14.4.2. * Hematology: Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count).

14.4.3. * Biochemistry: serum potassium, sodium, calcium, serum amylase, lipase **and LDH**;

Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;

Kidney function tests: urea, creatinine and uric acid.

14.4.4. Collection of any adverse event observed in the previous cycle and assignment of appropriate adverse events grade according to the NCI CTCAE Version 4.

14.4.5. Record all concomitant medication.

14.5. **Every second treatment cycle**

On day 1 of cycles 3, 5, 7 etc., or within 7 days before these dates:

14.5.1. T3, T4, TSH

14.5.2. Coagulation profile: **if medically indicated, according to institutional standard**

14.5.3. CA 15-3

14.5.4. Urinalysis: **proteins, glucose and blood using a dipstick.**

14.6. **Tumor assessments**

Tumor measurements according to RECIST 1.1 criteria (see Section 13) have to be done at baseline, at 12, 18 and 24 **weeks (+/- 1)**, then every 12 **(+/- 2)** weeks until progression, **or for a maximum of 24 months (see also Section 14.9)**:

14.6.1. Clinical and radiological tumor assessments will be done by CT scan or MRI.

14.6.2. Bone scan will be done if clinically indicated at the same time points.

14.7. **Cardiac evaluation**



- 14.7.1. Electrocardiogram (ECG): at baseline (≤ 42 days prior to treatment start) and at weeks 12, 24 etc. (every 12 \pm 2 weeks).
- 14.7.2. Echocardiography or MUGA scan: at baseline (≤ 42 days prior to treatment start), and at weeks 12, 24 etc. (every 12 \pm 2 weeks).
- 14.7.3. Cardiac enzymes BNP and Troponin: at baseline (≤ 42 days prior to treatment start), then every 12 (\pm 2) weeks until end of treatment.

14.8. **After discontinuation of trial treatment**

Trastuzumab can be continued in case of MK-3475 cessation due to toxicity. Similarly, MK-3475 can be continued if trastuzumab has been ceased, except in the case of cardiac dysfunction where both drugs must be stopped.

End of treatment visit **within 30 days of the last dose of trial treatment. If the decision to stop trial treatment comes more than 30 days after the last dose, this visit should be done as soon as possible.**

- 14.8.1. Physical examination, vital signs, ECOG Performance Status, and body weight.
- 14.8.2. Collection of any adverse event and assignment of appropriate adverse events grade according to the NCI CTCAE Version 4.
- 14.8.3. Record all concomitant medication.
- 14.8.4. Hematology: Hemoglobin, platelet count, white blood cell count including differential (absolute neutrophil count).
- 14.8.5. Biochemistry: serum potassium, sodium, calcium, serum amylase, lipase **and LDH**;
Liver function tests: albumin, total bilirubin, ALT, AST, ALP, GGT;
Kidney function tests: urea, creatinine and uric acid



- 14.8.6. T3, T4, TSH
 - 14.8.7. Coagulation profile: **according to institutional standard**
 - 14.8.8. If not done in the 30 days prior to this visit, clinical and radiological tumor assessments by CT scan or MRI.
 - 14.8.9. If not done in the 30 days prior to this visit, tumor measurements according to RECIST 1.1 criteria for determination of response (see Section 13).
 - 14.8.10. CA 15-3
 - 14.8.11. If treatment discontinued prior to progression, take two biopsies for translational research from a readily accessible lesion. Store one biopsy at -80°C and the second biopsy as a formalin-fixed, paraffin embedded block until shipment to the central lab.
 - 14.9. Follow-up prior to documented disease progression**
 - 14.9.1. **Patients stopping treatment early (prior to maximum treatment duration of 24 months), for any reason other than progression, will have a visit 12 (+/- 2) and 24 (+/- 2) weeks after stop of treatment.**
 - 14.9.2. **Patients that progress and stop treatment as well as patients taking treatment all the way to 2 years will have no further visits after the end of treatment visit; physical examination, vital signs, ECOG Performance Status, and body weight.**
 - 14.9.3. Collection of any adverse event **having occurred up to 30 days after last dose of trial treatment**, and assignment of appropriate adverse events grade according to the NCI CTCAE Version 4.
 - 14.9.4. Record all concomitant medication **up to 30 days after last dose of trial treatment.**
 - 14.9.5. **Electrocardiogram (ECG)**
 - 14.9.6. **Echocardiography or MUGA scan.**
 - 14.9.7. Clinical and radiological tumor assessments by CT scan or MRI.
 - 14.9.8. Bone scan if clinically indicated.
 - 14.9.9. Tumor measurements according to RECIST 1.1 for determination of response.
- If feasible, as soon as all trial treatment has been stopped or at PD, whichever occurs first, a biopsy should be taken (see 14.10.3).



14.10. At progression

14.10.1. Progressive disease is required to be confirmed 4 to 6 weeks later by the same imaging procedure.

14.10.2. Tumor measurements according to RECIST 1.1 for determination of progression

14.10.3. If not done already at time of discontinuation of trial treatment, take two biopsies for translational research from a readily accessible lesion. Store one biopsy at -80°C and the second biopsy as a formalin-fixed, paraffin embedded block until shipment to the central lab.

Note: After cessation of treatment, patients will have an End of Treatment visit as described in Section 14.8 and will afterwards be followed in clinic until confirmed progression or 24 weeks after stop of treatment, whichever occurs first.

15. Biological evaluations

15.1. Introduction and overview

Tumor tissue taken prior to start of treatment will be used to define biomarkers that could identify patients with a high chance of responding to MK-3475 combination with trastuzumab therapy. Biopsies at time of treatment discontinuation (usually at progression) will be used to understand resistance and response mechanisms.

Therefore, eligible patients should have lesions potentially suitable for serial biopsies. Patients will be asked to undergo biopsies pre-therapy (unless a biopsy was previously taken from a metastatic or loco-regional lesion) and on progression or at trial treatment discontinuation (and if possible, also from responders who stop trial treatment without progression). Lesions at time of end of trial treatment, usually at progression, can be exempt from repeat biopsy if too small (<1cm), or too dangerous. Tissue from the archival primary tumor (if breast tumor was removed at primary surgery) will also be requested; however, patients do not wait to enter the trial while the archival tumor is being obtained.

Biopsies from bone or bone marrow are not acceptable for screening.

The following tumor tissue samples will be taken and shipped to the central laboratory:

Table 8 Tumor tissue samples

Sample	Timepoint for submission	required	Purpose
Pre-treatment FFPE tumor block (including corresponding pathology report)	At screening; from biopsy of advanced disease , newly taken (preferred) or obtained ≤ 1 year prior to enrollment ¹	Mandatory	<ul style="list-style-type: none">- Central testing HER2 and PD-L1 for eligibility- Tumor infiltrating lymphocytes- DNA extraction for deep sequencing- ER/PgR status



Sample	Timepoint for submission	required	Purpose
FFPE tumor block from primary surgery (including corresponding pathology report)	At enrollment	Required, if available	- Central testing HER2 and PD-L1 - Tumor infiltrating lymphocytes - DNA extraction for sequencing
Pre-treatment fresh frozen tumor biopsy(+ path report)	Take at enrollment, from pre-treatment biopsy or primary surgery . Bank locally and submit upon request	Required, if available	- DNA extraction for sequencing - RNA extraction for RNA-seq
Post-treatment FFPE tumor block (+ path report)	A re- biopsy at time of progressive disease or end of all tx if ended prior to PD is strongly recommended	Required, if feasible	- Central testing HER2 and PD-L1 - Tumor infiltrating lymphocytes - DNA extraction for sequencing
Post-treatment fresh frozen tumor biopsy (+ path report)	From lesion biopsied at time of progressive disease or end of all tx if ended prior to PD. Bank locally and submit upon request	Required, if feasible	- DNA extraction for sequencing - RNA extraction for RNA-seq

¹ If central evaluation of archival tissue from a previous biopsy obtained ≤ 1 year from time of trial enrollment yields a negative test result, a new biopsy may be taken and submitted for central evaluation.

Table 9 Blood samples for all patients

Purpose	Type of sample	Cycle 1	Cycle 3	Cycle 5, 8, 11, ... (every 3 cycles)	30 days after treatment stop
cpDNA	Plasma from 8-10 mL whole blood	Before dose	Before dose	Before dose	Yes
Confirmation of somatic nature of mutations found in tumor biopsies	10 mL whole blood	Before dose			

15.2. Submitting pathology material to IBCSG

All FFPE blocks and copies of Pathology Reports must be marked with the IBCSG Patient ID number. Please erase or black out any other identifiers like name or date of birth. Refer to the IBCSG website for additional FFPE specimen shipping recommendations.

All FFPE samples should be submitted within 4 weeks after being available. The biopsy used for central determination of HER2 and PD-L1 must be submitted prior to enrollment.

Mailing address for the FFPE blocks and Pathology Reports:



IBCSG Central Pathology Office
European Institute of Oncology, EIO
Division of Pathology
Via Ripamonti 435
20141 Milano, Italy
E-mail: pathology.ibcsg@ieo.it

NOTE: Pathology Reports must also be submitted to DataFax via fax or DFsend.

Fresh frozen tumor blocks will be stored locally at -80°C and collected later.

15.3. Central testing of HER2 and PD-L1

The IBCSG Central Pathology Office at European Institute of Oncology, Milan, Italy, will determine the HER2 status for submitted biopsies. Five 5 µm sections (FFPE) will be sent to the certified lab for PD-L1 determination by IHC.

HER2 status will be quantified by copy number and FISH ratio.

The certified lab is [Quintiles, Scotland, UK](#).

15.4. Banking biological material

All FFPE blocks will be banked in the IBCSG Tissue Bank, to have available for Translational Research, and tumor DNA will be extracted and cryopreserved (to avoid DNA degradation in FFPE). All biological material will be logged in the IBCSG Pathology Material Tracking System /Translational Research Information Management System and banked in the IBCSG Tissue Bank.

The use of the biological material for not yet specified future research will be under the auspices of the IBCSG Biological Protocols Working Group. As part of the Informed Consent process, patients are asked to agree to donate their sample for not yet specified future research.

15.4.1. FFPE

Remaining FFPE tumor blocks from the metastatic lesion will be stored in the IBCSG tissue biobank (pre-treatment and at treatment discontinuation). See Section 15.2 for shipping address and also the [“IBCSG 45-13 PANACEA Pathology Procedures Manual”](#). [FFPE material from patients screened negative for PD-L1 will be kept in the biobank as well.](#)

Archived primary tumor material is also requested from the patient.

15.4.2. Frozen tumor biopsies

We request that one to two frozen tumor biopsies are taken, prior to start of treatment and at treatment discontinuation, and stored in liquid nitrogen or O.C.T. (Optimal Cutting Temperature) Compound – the latter is preferred. Bank the fresh frozen tumor blocks locally and submit upon request. [The fresh frozen biopsies will be centralized at IBCSG at the end of the trial, shipped in one single batch. The IBCSG will coordinate this temperature](#)



controlled transfer. Please refer to the “**IBCSG 45-13 PANACEA Pathology Procedures Manual**” for more details on the OCT or on alternative methods, such as Snapfrost.

15.4.3. Blood samples

At the end of the trial, blood and plasma samples will be collected by courier and sent to:

Harriet Johansson
Lab Senior Assistant
Division of Cancer Prevention and Genetics
European Institute of Oncology
Via Ripamonti 435, 20141 Milan, Italy

Please consult the “**Blood Sample Logistics Manual**” for details.

15.5. Translational research

Given that this is a proof-of-concept trial exploring a new therapeutic area for breast cancer, a complete collection of biological materials is requested in order to be able to comprehensively define the molecular and immune landscape of these tumors. The goals of the translational research will be to determine mechanisms and biomarkers for MK-3475 efficacy and resistance.

Planned correlative studies:

15.5.1. Biomarkers of PD-1/PD-L1

Quantification of PD-L1 and PD-1 expression will be done by a Merck CLIA-certified IHC test in a central lab. This marker will be performed in the primary tumor and unresectable loco-regional or metastatic biopsies pre-treatment and at time of trial treatment discontinuation.

15.5.2. Multiparametric IHC

Spatial association of PD-1+ tumor infiltrating lymphocytes (TILs) and PD-L1+ cells (tumor and myeloid cells) suggests “induction” of PD-L1. Interferon-gamma production by antigen-specific PD-1+ CD8+ T-cells is hypothesized to drive local intra-tumoral upregulation of PD-L1 on adjacent tumor and myeloid cells, leading to a “stalled Cytotoxic T Lymphocyte (CTL)” response which may be predictive of response to MK-3475 therapy. By assessing both of the required elements, (i.e., PD-L1 expressing cells and PD-1+ T-cells) an IHC assay may be a better predictor of response than PD-L1 expression alone.

15.5.3. Characterization of tumor infiltrating lymphocytes

Quantification of tumor infiltrating lymphocytes will be performed independently by two pathologists (Giuseppe Viale and Carsten Denkert). The percentage infiltration in the adjacent stromal areas as previously described [3,4] will be recorded. This assessment will be performed in the primary tumor and metastatic biopsies pre-treatment and at time of trial treatment discontinuation.

Agreement between assessments by pathologists will be analyzed.



Objective responses will be assessed as a function of TILs adjusted for other clinico-pathological factors, the hypothesis being that high levels of TILs will be associated with a higher rate of objective responses in this trial.

15.5.4. Characterization of circulating plasma DNA (cpDNA)

Plasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden. This information may be useful as a non-invasive way of monitoring advanced disease using cancer genetic alterations (mutations, rearrangements) that are specific to the individual's tumor. This information may allow us to track and monitor tumor dynamics during the disease course as well emergence of new clones (i.e., resistance mechanisms). Individual mutations may be assessed using technologies such as Sequenom Mass Spectrometry or deep sequencing.

Plasma needs to be stored at -80°C until shipment. See Section 15.1 for an overview of sampling timepoints.

For cpDNA extraction, plasma will be thawed at ambient temperature and cpDNA extracted from 2 mL of plasma using a QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions, with the following modifications: for each 2 mL sample of plasma, an additional centrifugation step (16000 g, 5 min, RT) was added before the extraction procedure in order to eliminate cellular debris from the plasma. At the end of the procedure, the DNA will be eluted in 100 μL of AE elution buffer. DNA concentration will be measured with fluorescent staining, using the Quant-iT™ Pico-Green® double stranded DNA (dsDNA) Assay Kit (Invitrogen, Carlsbad, CA) and the SynergyHT microplate reader (Biotek).

15.5.5. Sequencing of tumor biopsies (DNA and RNA)

Frozen and FFPE biopsies will be retained for deep sequencing using next generation technologies at the completion of the trial. Nucleic acids will be extracted according to standard protocols. Sequencing will be performed using the Illumina Hi-Seq, though the technology is improving at a rapid rate.

The aim of the sequencing is to understand the molecular landscape (mutations, rearrangements and copy number changes) associated with HER2-positive, PD-L1 expressing tumors as well as response or resistance to the trial therapy. Specific mutational patterns may be associated with increasing PD-L1 expression or lack of TILs (i.e., chromosomal instability, certain somatic mutations) which could imply a means by which the tumor escapes and subverts host immunity. This may be amenable to targeting with another compound. Similarly, tumors may upregulate specific pathways in order to avoid immune destruction after the PD-1 pathway has been inhibited. The presence of intra-tumoral heterogeneity will be investigated for in tumor biopsies and if this correlates with tumor responses.

Paired metastatic lesions will be compared for the changes that occur during progression and resistance to the MK-3475/trastuzumab combination. This will be also compared with the primary tumor. New data are emerging that suggest we can define certain tumor types as



being ‘hypermuted’. There is a potential that this hypermuted state may correlate with response to MK-3475 therapy, and/or that the converse, ‘hypomuted’ state may correlate with non-response.

If enough RNA is also able to be extracted, gene expression of these tumors will be evaluated using a genome-wide, deep sequencing technology (RNA-seq). Specific markers of immune response (immunosuppression as well as activation) can be then assessed for changes during the treatment course. For example, the expression of Tregs (FOXP3) as well as IDO1, an immunosuppressive soluble factor may be increased at progression. Gene signatures that are published in the literature representing various aspects of immunity could also be assessed for their change during treatment.

Whole blood will be collected at baseline prior to treatment. The sample will be processed and stored at -80°C. It will be used to confirm the somatic nature of any interesting mutations found. This will not be used for analysis of germline abnormalities.

DNA and RNA will be analyzed to attempt to define a gene set critical for clinical response to MK-3475. The hypothesis to be tested is that MK-3475 responders will exhibit a “stalled Cytotoxic T Lymphocyte (CTL)” response within the tumor reflected in the physical proximity between PD-1 and PD-L1 expression and the presence of an aborted (e.g., weak but discernible) interferon-gamma transcriptional program will be detectable by profiling analyses. Significant alterations identified with these technologies can also be evaluated using cpDNA.

15.5.6. Further exploratory biomarkers

Merck will perform IHC evaluation, minimally to include PD-1 and PD-L1 IHC, as well as Nanostring-based gene expression analysis. This can be accomplished with 10-20 unstained 4-5 micron slides. Merck will deliver the results to IBCSG.

15.5.7. Not yet specified translational research

Translational research proposals not outlined in this protocol will be assessed by a trial-specific research committee for merit and feasibility.

Biomarkers that are published in the future and considered to be of relevance can then also be assessed in the context of this trial.

For example, an anti-HER2 adaptive response could be assessed and monitored in plasma samples should a suitable antibody be found.

16. Data submission

We will conduct the trial according to the ICH Good Clinical Practice (GCP) guidelines. Keeping accurate and consistent records is essential to a cooperative trial. **Forms need to be submitted within 1 week of visit (unless otherwise indicated in table below).** The following forms are to be submitted at the indicated times by the participating institutions for each patient:



16.1. Case report forms schedule

<i>Forms</i>	<i>Description/Name</i>	<i>Forms Submission</i>
Registration		<i>ALL data should be completed in iDataFax (iDF) (unless otherwise specified)</i>
Informed Consent Form	Consent to participation in clinical trial	Obtain before registration and keep with patient records as documentation (hard copy only).
45-A1	Confirmation of Registration Form	Complete in iDF after you have registered the patient in the IBCSG Registration/Randomization System
Enrollment		
45-A2	Confirmation of Enrollment Form	Complete in iDF after you have enrolled the patient in the IBCSG Registration/Randomization System.
Baseline		
45-H	History Form	Complete in iDF within 1 week of enrollment.
Path. Report	Pathology Report	Submit Report(s) of unresectable loco-regional and/or metastatic lesion to DataFax System within 1 week of enrollment via fax or DFsend.
45-BAE	Baseline Adverse Events and Symptoms Form	Complete in iDF within 1 week of enrollment.
45-CE	Cardiac Evaluation Form	Complete in iDF within 1 week of enrollment.
45-CCM	Concomitant Medication Form	Complete in iDF each time a medication is started, amended, or ended, including medications taken within 28 days prior to start of treatment (excluding prior treatment for primary/metastatic breast cancer).
45-TEV-B	Tumor Evaluation Baseline Form	Complete in iDF within 1 week of enrollment.
45-FFBI-PT	Fresh Frozen Biopsy Information – Pre-Treatment Form	Complete in iDF within 1 week of enrollment for biopsy taken ≤ 28 days prior to treatment start.
During trial treatment		
45-CYC	Treatment Cycle Form	Complete in iDF at the end of each cycle until treatment stops.
45-DLT	Dose Limiting Toxicity Form (during cycle 1 only)	Complete in iDF for cycle 1 immediately if DLT is observed , otherwise at end of cycle 1 for all patients in dose finding phase 1b part of the trial.
45-AE	Adverse Events Form	Complete in iDF at the end of cycle 1 of trial treatment. At the end of each subsequent cycle, review the original AE Form in iDF. Update AEs if needed and add any new AEs.
45-CCM	Concomitant Medication Form	Review the original CCM Form in iDF at the end of each cycle. Update any current medications if needed and add new medications taken.
45-BSC	Blood Sample Collection Form	Complete/Update in iDF at each blood sample collection.
45-FFBI-PRO	Fresh Frozen Biopsy Information – Progression Form	Complete in iDF at progression if re-biopsy is taken.
45-CE	Cardiac Evaluation Form	Complete in iDF every 12 weeks while on treatment
45-TEV	Tumor Evaluation Form	Complete in iDF at weeks 12, 18 and 24, then every 12 weeks while on treatment . A confirmatory TEV Form should also be submitted 4-6 weeks after CR, PR and PD



<i>Forms</i>	<i>Description/Name</i>	<i>Forms Submission</i>
<i>Follow Up (after discontinuation of trial treatment)</i>		
45-EoT	End of Treatment Form	Complete in iDF 30 days after ALL protocol treatment stops.
45-E	Follow up Form	Complete in iDF 12 and 24 weeks after all protocol treatment stops, if patient discontinued trial treatment prior to documented progression.
45-TEV	Tumor Evaluation Form	<i>If</i> patient discontinued trial treatment prior to documented progression: every 12 weeks until progression.
45-AE	Adverse Events Form	Review and submit the AE Form in iDF 30 days after the last dose of trial treatment. Update AEs if needed and add any new AEs.
Path. Report	Pathology Report	Submit Report of re-biopsy post-progression (if available) to DF via fax or DFsend.
<i>Event Driven</i>		
45-SAE/ECI-A	Serious Adverse Event/Event of Clinical Interest Form - Initial report	Complete in iDF within 24 hours of the SAE or ECI. If iDF is not available, fax the form within 24 hours to DataFax.
45-SAE/ECI-B	Serious Adverse Event/Event of Clinical Interest Form B - Follow-up report	Complete in iDF within 15 days of the initial report (45-SAE/ECI-A). If event is not resolved in 15 days, complete 45-SAE/ECI-B again at the time of resolution.
45-PREG	Pregnancy Form	Complete in iDF if a patient becomes pregnant on trial treatment or within 7 months of last dose. Complete the second section of the form at the outcome of pregnancy.
45-COC	Change of Consent Form	Complete in iDF if there is any change in patient's consent to participate in the trial, see Section 18.5.2.
45-Death	Death Form	Complete in iDF if a patient dies.

The Data Managers' Manual for this trial contains instructions for submitting forms using the iDataFax system.

16.2. Signing and submitting forms

An Authorization Log (see Section 16.5) should be completed at each Participating Center to identify the persons who are authorized to complete CRFs.

CRFs should be completed on-line in iDataFax. Reports (lab, pathology, etc.) and any other non-CRF data will need to be sent to the DataFax system via fax or DFsend. Full instructions on submitting forms will be available on the IBCSG website (www.ibcsg.org). Also available on the website is a list of fax numbers that are available for faxing CRFs.

16.3. Data management

Data collected in this trial will be submitted to the IBCSG Data Management Center in Amherst, NY, USA. The Data Management Center will process the data and will generate queries and forms requests. The Data Quality Control Office will oversee overall data submission and query resolution. The IBCSG Coordinating Center in Bern, Switzerland will provide medical review and summary of SAEs. The IBCSG Statistical Center in Boston, MA, USA will perform the data analysis.



16.4. Investigator Site File

Each Participating Center should keep documentation about this trial in an Investigator Site File, which should include the following documents:

- Protocol and appendices
- Amendments
- Signed Protocol Signature Pages
- Sample CRFs including blank SAE Forms
- Data Managers' Manual
- Obvious Corrections Document and Signature Page
- Randomization Manual
- iDataFax (iDF) Manual
- Drug Supply Manual
- Latest version of MK-3475 Immune-Related Adverse Event Guidance document
(Only for Centers activated to original protocol, who have received this document)
- Manual for Blood Sample Logistics
- Pathology Procedures Manual
- Slotting Procedures document
- Patient information and Informed Consent templates approved by Ethics Committee
- Investigator's Brochure and updates
- Ethics Committee (and Health Authority, if applicable) approval of protocol, Patient Information Sheet and Informed Consent, amendments
- Ethics Committee review of SAE, Investigators' alert, and other documents
- Correspondence with Ethics Committee and Health Authority (if applicable)
- Certificate of clinical trial insurance
- Agreement with IBCSG
- Center Activation email from DMC
- Correspondence with IBCSG Coordinating Center, Data Management Center
- SAE Reports sent from IBCSG Data Management Center
- Normal laboratory values/reference ranges
- Laboratory Certifications
- CV of Principal Investigator and Co-Investigators, GCP certificates
- Trial Training Certificates issued by IBCSG Center Training Office
- Authorization Log
- Patient Identification Log (see Section 16.6)
- Drug Accountability Log (including certificates of destruction if applicable)
- Temperature logs
- ICH GCP guidelines/Declaration of Helsinki and updates
- Audit certificates / monitoring reports



16.5. Authorization Log

The Principal Investigator (PI) should identify the other members of the Clinical Trial Team who are supervised by the PI and approved to provide information in CRFs, queries, etc. Instructions for completing the Authorization Log can be found in the “*Authorization Log Manual*”, posted on the IBCSG website.

16.6. Patient Identification Log

No patients’ names should be used in CRFs or any other documentation transmitted to IBCSG central offices. The only item used to identify a patient is the patient ID number. It is therefore imperative that the local data manager keep an identification log for all patients entered in this trial including:

- Patient's name
- Patient ID issued by the Registration/Randomization System
- Date of birth

Other items that could be included are date of enrollment and dose.

17. Statistical considerations

This is a multi-center, open-label phase Ib/II trial of the combination of the anti-PD-1 monoclonal antibody, MK-3475, with trastuzumab **with primary objectives for** patients with confirmed HER2-**positive** and PD-L1-expressing, unresectable loco-regional or metastatic breast cancer who have experienced progression with previous trastuzumab-based therapy. The primary objective of the dose escalation (phase Ib) portion of the trial is to determine the RP2D of MK-3475 in combination with standard dose trastuzumab. The **primary objective of the phase II portion of the trial** will evaluate the efficacy and safety of the drug combination.

The secondary objective will explore the efficacy and safety of the drug combination in a parallel cohort of patients with confirmed HER2-positive, PD-L1 negative disease.

17.1. Primary and Secondary Endpoints

Phase Ib:

Primary Endpoint: The incidence of dose-limiting toxicity of MK-3475 in combination with standard dose trastuzumab (defined in Section 10.2.1).

Phase II:

Primary Endpoint: Objective response (confirmed CR or PR as best overall response) according to RECIST criteria (Version 1.1) as defined in Section 17.7.1.

Secondary Endpoints:



- 17.1.1. Safety and tolerability according to CTCAE version 4.0.
- 17.1.2. Disease control (DC) defined as best overall response of confirmed CR, PR, or SD lasting for 24 weeks or longer, measured from the start of trial treatment until first documentation of progressive disease.
- 17.1.3. Duration of response (DoR) is defined among patients with objective response (confirmed CR or PR as best overall response) as the interval between dates of first documentation of objective response and first documentation of progressive disease. In the absence of documented progressive disease, follow-up will be censored at date of last disease assessment.
- 17.1.4. Time to progression (TTP) defined as the interval between the dates of the start of trial treatment and first documentation of progressive disease. In the absence of documented progressive disease, follow-up will be censored at date of last disease assessment.
- 17.1.5. Progression-free survival (PFS) is defined as time from start of trial treatment until documented disease progression or death, whichever occurs first. Patients with new non-breast cancer malignancy must continue to be followed for progression of the original breast cancer. For patients without progression, follow-up will be censored at the date of last disease assessment without progression, unless death occurs within 12 weeks following the date last known progression-free, in which case the death will be counted as a PFS event.
- 17.1.6. Patients who discontinue or initiate non-protocol treatment prior to documented disease progression will be followed for disease progression.
- 17.1.7. Overall Survival (OS) is defined as the time from start of trial treatment to death from any cause. For patients who are lost to follow-up or who have no documentation of death at the time of final analysis, follow-up will be censored at the date of last assessment of vital status.

17.2. Definitions of Trial Populations

Safety population: All patients receiving at least one dose of MK-3475 will be included in assessments of safety.

Evaluable population: All patients receiving one or more doses of MK-3475 *and* trastuzumab will be considered evaluable. In the phase Ib study, patient data will be summarized according to the assigned MK-3475 dose.

The phase II study population will be comprised of evaluable patients enrolled in the two parallel cohorts. The assessment of the primary efficacy objective will be based on patients with PD-L1 expressing disease. The secondary objective will be based on patients with PD-L1 negative disease.

17.3. Design and Sample Size Determination

Phase Ib: Determination of the recommended dose for the phase II part of the trial will be based on a standard 3+3 dose-escalation design. See Section 10.2 for the definition of DLT, the dose escalation scheme, and dose levels. A patient in the phase Ib portion of the trial will



be replaced if determination of DLT cannot be adequately assessed because of rapid disease progression or if treatment and/or follow-up is stopped during the first cycle of therapy for reasons other than toxicity. Patients who do not receive trastuzumab in the first cycle of therapy due to an infusion reaction to MK-3475 will also be replaced and will not be counted as having a DLT.

The following table summarizes the probability that the dose will be escalated (i.e., that 0 in 3 patients or 1 in 6 patients experiences DLT) given the true but unknown rate of DLT in cycle 1.

True, but Unknown, DLT Rate	Probability of Dose Escalation
10%	91%
20%	71%
30%	49%
40%	31%
50%	17%
60%	8%

Phase II:

PD-L1 Expressing: A Simon optimal two-stage design [55] was used to estimate the sample size for the **primary efficacy objective of the** phase II trial.

The null hypothesis of a true objective response rate (ORR) of 7% will be tested against a one-sided alternative objective response rate of 22%. The rationale for the null and alternative hypotheses is based on several trials. In a comparable trial population of patients progressing on trastuzumab therapy, single-agent lapatinib yielded an ORR of 6.9% and clinical benefit rate (CBR) of 12% compared with lapatinib and trastuzumab (ORR 10.3%, CBR 24.2%) [56]. Lapatinib has recently been shown to be inferior to trastuzumab in a head-to-head comparison in the metastatic setting, and the single-agent arm was dropped due to an inferior outcome in the phase III, ALTTO adjuvant trial. In a single-arm, multi-site, phase II trial of combination trastuzumab and pertuzumab in patients progressing on previous trastuzumab, an ORR of 24.7% was observed with a CBR of 50% [57]. Subsequently, this combination was approved by the FDA in the first-line metastatic setting with docetaxel [58] and is currently being evaluated in an adjuvant, phase III trial in 3800 women (APHINITY). This trial was conducted in a time where patients were generally not exposed to other anti-HER2 agents such as lapatinib and trastuzumab-emtansine, hence this objective response rate is unlikely to be seen in today's population where all metastatic patients receive a second anti-HER2 therapy prior to receiving investigational therapy. This is supported by recent data from the combination of BKM120 (an oral pan-class I PI3K inhibitor) and trastuzumab in patients with advanced or metastatic breast cancer who progressed while on trastuzumab, reported an ORR of 8% and CBR of 49% [59]. However, we have selected the alternative objective response rate to be slightly less than the reported response rate of the combination of trastuzumab and pertuzumab given that a reasonable ORR should be seen in order to move into the phase III setting.



The optimal two-stage design will be based on a total of 40 evaluable patients. Seventeen (17) evaluable patients will be enrolled in the first stage, and if 1 or fewer objective responses are observed in 17 patients, then enrollment will stop completely. If there are 2 or more objective responses, then an additional 23 patients will be enrolled, for a total of 40 evaluable patients. The null hypothesis will be rejected if a total of 6 or more objective responses are observed in 40 evaluable patients. This design yields a type I error rate of 0.05 (target type I error of 0.05) and power of 85% (target type II error of 0.15) when the true objective response rate is 22%. If the null hypothesis is true, the probability is 0.66 that enrollment will stop at the end of the first stage. **Enrollment will pause at the end of the first stage to allow for an early assessment of response if two objective responses have not yet been observed among the first 17 evaluable patients.**

PD-L1 Negative: A PD-L1 negative cohort is included to provide evidence of objective response and inform subsequent drug development in patients selected or unselected for PD-L1 status. A single-stage design with an enrollment of 15 patients will be used to compare a null response rate of 1% with a desirable response rate of 20%. The cohort size was selected to yield a very high probability of not missing a response signal if one exists. The decision rule is based on zero responses: the drug combination would not be considered worthy of further investigation if no patients respond. If the true response rate is 20%, then the probability that zero responses would be observed in 15 patients is 0.035 (target type II error of 0.05). Therefore, there would be less than 4% chance of missing a true 20% response rate if the alternative hypothesis is true. The type-I error for the design is 0.14.

Concurrent with the first-stage review of efficacy in the PD-L1 expressing cohort, a detailed safety review will be conducted, as described in Section 11.5 and in Section 17.4. The safety review will also include data from patients in the secondary cohort with PD-L1 negative disease who are enrolled prior to the 17th evaluable patient with PD-L1 expressing disease.

17.4. Safety Monitoring

The safety review will be concurrent with the first efficacy review of **objective response** that is specified by the Simon two-stage design. **This review will be based on 17 evaluable patients with PD-L1 expressing disease from the first stage of the two-stage design, and patients in the secondary cohort with PD-L1 negative disease who are enrolled prior to the 17th patient with PD-L1 expressing disease.** Review of these safety data will be conducted by the trial Steering Committee (see Section 19.2) and an independent IBCSG Data and Safety Monitoring Committee (DSMC, see Section 19.3) and reported within one month. Based on the review, the DSMC may advise that the trial be suspended for additional review and possible dose modification; however, the final decision regarding suspension or dose modification will be made by the trial Steering Committee. After this initial safety assessment, toxicity and adverse event data will be evaluated as part of the semi-annual trial review by the DSMC.

The total number of patients included in the safety review will be between 17 and 32 patients and will depend upon the number of patients enrolled in the secondary PD-L1 negative cohort at the time of the assessment. The decision rules and probabilities presented



below are based on 17 patients with PD-L1 expressing disease; however, these will be adjusted based on the actual number of patients in the safety analysis to provide comparable operating characteristics.

As part of the early safety monitoring, we will focus particular attention on cardiac events (CE) of grade 3 or higher (Section 11.5). If 3 or more of the first 17 patients experience CE, then the trial enrollment may be suspended. Table 10 summarizes the probability of observing 3 or more CE based on the first 17 patients for various underlying true CE probabilities. If the true probability of cardiac toxicity is 18% or higher the probability is greater than 60% that 3 or more patients will be observed with CE at the time of the early safety monitoring.

Table 10 Operating characteristics of early cardiac monitoring

True Incidence of Cardiac Toxicity	Probability of Observing Three or More Patients with CE among First 17
1%	< 0.01
3%	0.01
5%	0.05
10%	0.24
15%	0.48
18%	0.61
20%	0.69
30%	0.92

All reported AEs will also be summarized as part of this safety review. For a sample of 17 patients, there is high probability of observing at least one event if a toxicity has an incidence of at least 9%. If the true incidence of an unexpected or severe toxicity is 9% or greater, the probability is at least 0.80 that one or more patients out of 17 will experience the toxicity during the early safety monitoring period. If the true incidence of unexpected or severe toxicity is 3% or less, the probability is 0.40 or less that at least one patient of 17 will experience the toxicity during the early safety monitoring period. Table 11 summarizes the probabilities of observing at least one patient with a severe or unexpected toxicity during the initial safety review for a range of true incidence rates.

Table 11 Operating characteristics of early safety monitoring

True Incidence of Unexpected or Severe Toxicity	Probability of Observing One or More Patients with Toxicity among First 17
1%	0.16
3%	0.40
5%	0.58
8%	0.76
9%	0.80
10%	0.83
15%	0.94



17.5. Screening and Enrollment

The total enrollment of this phase Ib/II trial will be between 6 and 61 evaluable patients. The minimum would occur if the combination of MK-3475 and trastuzumab proved too toxic at **body weight-based** dose levels 1 and -1 and the trial was stopped during the dose escalation phase. A maximum enrollment of 61 patients reflects two complete dose escalation levels of 6 patients each **and 55 patients enrolled during phase II**. Under the assumption that approximately **two thirds** of patients will be found ineligible upon screening, it is estimated that **about 120** patients will be screened to obtain the maximum sample of 61 evaluable patients.

17.6. Analysis of Demographics/Baseline Characteristics

Demographic and other baseline characteristics will be summarized for all patients by study population (Ib or II (**PD-L1 expressing, PD-L1 negative**)), and by dose level for patients in the phase Ib study. Demographic and baseline characteristics of patients who are replaced (Section 17.3) will be summarized in a separate category. Summaries of continuous demographic/baseline variables, including age, weight, and vital signs, will be presented as N, mean, standard deviation, median, quartiles, and minimum and maximum values. For categorical variables, such as ER status, the number and percentage of patients will be used.

17.7. Efficacy Analysis (Phase II)

Primary **and** secondary **endpoints**, and correlative objectives of the phase II portion of the trial will be based on the phase II population, unless otherwise stated. **Efficacy endpoints will be analyzed separately by PD-L1 cohort. Safety endpoints will be summarized in the aggregate for all 55 patients (PD-L1 expressing and PD-L1 negative).**

17.7.1. Primary Endpoint

At the time of each restaging, patients will be classified as achieving complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), or non-evaluable for response according to RECIST (Version 1.1) criteria. Objective response will be determined by the best overall confirmed response designation recorded between the date of first dose of trial therapy and the date of objectively documented disease progression or cessation of trial therapy, whichever occurs first. For patients without documented progression or cessation of trial therapy, all available response designations will contribute to the objective response determination.

- **For the primary objective, the proportion of patients with PD-L1 expressing disease with an objective response will be presented with a two-sided 90% confidence interval calculated using the method of Atkinson and Brown [60], which allows for the two-stage design.**
- **For the secondary objective, the proportion of patients with PD-L1 negative disease with an objective response will be presented with a two-sided 90% exact binomial confidence interval. For a sample of 15 patients, the confidence interval will be no wider than 0.46.**



17.7.2. Secondary Endpoints

Safety and Tolerability: All adverse events recorded during the trial will be summarized for the safety population. The incidence of events that are new or worsening from the time of first dose of treatment will be summarized according to system organ class and/or preferred term, severity (based on CTCAE grade), type of adverse event, and relation to study treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, and type of adverse event.

Disease Control Rate: The proportion of patients with disease control will be presented with two-sided 90% confidence intervals estimated using exact binomial methods. Based on the sample size of 40 patients with PD-L1 expressing disease, the confidence interval will be no wider than 0.32; for the sample of 15 patients with PD-L1 negative disease, the confidence interval will be no wider than 0.46.

DoR, TTP, PFS and OS: The distributions of duration of response (DoR), time to progression (TTP), progression-free survival (PFS), and overall survival (OS) will each be summarized using the product-limit method of Kaplan-Meier. The two PD-L1 expression cohorts will be summarized separately. Median times for each endpoint will be presented with two-sided 90% confidence intervals estimated using log(-log(survival)) methodology. Kaplan-Meier estimates of TTP and PFS at 6 or 12 months after treatment initiation will also be presented with two-sided 90% confidence intervals.

17.8. Correlative Objectives

The analysis of correlative objectives will be based on the combined cohorts of evaluable patients with PD-L1 expressing and PD-L1 negative breast cancer enrolled in the phase II portion of the trial. Stratified analyses according to PD-L1 expression will also be conducted.

17.8.1. Tumor-Infiltrating Lymphocytes (TILs)

The relationship between tumor-infiltrating lymphocytes (TILs) and outcomes will be explored according to pre-treatment TIL percentages. It is hypothesized that higher levels of lymphocytic infiltration will be associated with better outcomes. Histopathologic evaluation of TILs will be performed independently by two pathologists and the mean value will be used for the analysis. Stromal lymphocytic infiltration will be defined as the percentage of tumor stroma containing infiltrating lymphocytes. TILs will be evaluated as previously described [3,4]. Bland-Altman analyses will be used to summarize agreement between pathologists.

To examine responses according to levels of TILs, the population will be divided retrospectively according to objective response or non-response. Pre-treatment percentages of stromal infiltrating lymphocytes will be summarized descriptively for the two response groups and compared using Wilcoxon rank-sum tests. If there are 9 responses and 46 non-responses, a Wilcoxon rank-sum test with a two-sided, 10% type I error will have 85% power to detect a difference in baseline lymphocyte percentage that is 1.06 times the common standard deviation. Visualization of the relationship between baseline TILs and the



distributions of TTP or PFS will employ Kaplan-Meier estimates stratified by lymphocyte-predominant breast cancer (LPBC) phenotype or median of the distribution of intra-tumoral or stromal percentages. LPBC phenotype will be defined as 50% infiltration of either stromal or intra-tumoral lymphocytic infiltration. Medians of the time-to-event endpoints will be shown with two-sided 90% confidence intervals; the distributions of TTP or PFS will be compared across TIL strata using the log-rank test.

Changes in TILs between baseline and progression/treatment discontinuation will be calculated (post-pre) for each patient and summarized descriptively. In addition, the correlation of TIL changes with measures of peripheral blood markers will be explored graphically, or by appropriate statistical methods based on data availability, to assess associations.

17.8.2. PD-L1 Expression Levels

The association between pre-treatment PD-L1 expression and outcomes will be explored. It is hypothesized that the upregulation of PD-L1 may allow cancers to evade the immune system; therefore, higher expression levels of PD-L1 would be associated with poorer outcomes. To assess the association of pre-treatment PD-L1 with response, the proportion of patients with objective response according to pre-treatment PD-L1 will be summarized with two-sided 90% exact, binomial confidence intervals. Visualization of the relationship between baseline PD-L1 expression and the distributions of TTP or PFS will employ Kaplan-Meier estimates stratified by PD-L1 expression. Medians of the time-to-event endpoints will be shown with two-sided 90% confidence intervals.

The relationship between PD-L1 expression and TIL expression will also be explored to better characterize the tumor microenvironment. Pre-treatment percentages of stromal infiltrating lymphocytes will be summarized descriptively according to PD-L1 expression and compared using Wilcoxon rank-sum tests. Descriptions of the immune repertoire of TILs will also be presented based on available data.

17.8.3. Estrogen Receptor (ER) Expression

The association between pre-treatment expression of ER and outcomes will also be explored as a secondary objective. Pre-treatment ER expression will be dichotomized as present ($\geq 1\%$ expression) or absent ($< 1\%$). The proportions of patients with objective response in each ER subgroup will be summarized with two-sided 90% exact, binomial confidence intervals. The distributions of TTP or PFS will be compared across the ER strata using the log-rank test. If the distribution of ER expression is not sparse, we will employ STEPP (Subpopulation Treatment Effect Pattern Plot [61]) analyses over the continuous range of baseline ER values to visualize the relationship between ER expression and the distributions of PFS or TTP.

17.8.4. Outcome According to Quantified HER2 Level

To examine responses according to pre-treatment levels of FISH ratio (HER2/CEP17) or HER2 gene copy number, the study population will be divided retrospectively according to objective response or non-response. Pre-treatment FISH ratios or HER2 copy numbers will



be summarized descriptively for the two response groups and compared using Wilcoxon rank-sum tests. If there are 9 responses and 46 non-responses, a Wilcoxon rank-sum test with a two-sided, 10% type I error will have 85% power to detect a difference in measure that is 1.06 times the common standard deviation. Visualization of the relationship between FISH ratio or HER2 copy number and the distributions of TTP or PFS will employ Kaplan-Meier estimates. FISH ratio or HER2 copy number data will each be divided into high/low groups at the medians of the respective distributions. The distributions of TTP or PFS will be compared across FISH ratio or HER2 copy number strata using the log-rank test; medians of the time-to-event endpoints will be shown with two-sided 90% confidence intervals.

17.8.5. Tumor dynamics

The pharmacodynamic effects of the combination of MK-3475 and trastuzumab on markers in peripheral blood and serum proteins will be assessed by summary statistics, and investigated graphically to explore patterns of change over time and differences according to treatment exposure. Associations between biomarker measures from peripheral blood or tumor biopsy and clinical outcomes will also be explored graphically.

17.9. Exploratory Objectives

The RECIST response category of partial response (PR) will be divided into two subcategories: moderate partial response (MPR: 30-59% uni-dimensional reduction) and very good partial response (VGPR: 60% or greater uni-dimensional reduction) to address the exploratory hypothesis that patients with VGPR will have longer duration of response than patients with MPR. Duration of response will be summarized using a conditional landmark analysis to address potential guarantee-time bias. The relevant landmark time will be established prior to any analyses of the data. Median durations of response for each subcategory will be presented with two-sided, 90% confidence intervals. A second analysis will include patients with CR as a third subcategory, if possible.

Correlative investigations between MPR/VGPR and pre-treatment ER, PD-L1, FISH ratio HER2 copy number, and TILs will also be conducted to investigate if levels of these biomarkers differ between MPR and VGPR responders.

18. Ethical aspects, regulatory approval, and patient informed consent

The Investigator will ensure that this trial is conducted in full conformance with the principles of the “Declaration of Helsinki” or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The trial must fully adhere to the principles outlined in “Guideline for Good Clinical Practice” ICH Tripartite Guideline (January 1997) or with local law if it affords greater protection to the patient. For studies conducted in the EU/EEA countries, the Investigator will ensure compliance with the EU Clinical Trial Directive (2001/20/EC).



18.1. Ethical Review Board/Ethics Committee

All protocols and the patient Informed Consent forms must have the approval of a properly constituted committee or committees responsible for approving clinical trials. The ERB/IRB written, signed approval letter/form must contain approval of the designated Investigator, the protocol (identifying protocol title and version number), and of the patient Informed Consent. Documentation of Ethics Committee approval(s) must be sent to the IBCSG Data Management Center prior to enrollment of the first patient. The IBCSG Ethics Committee also approves the protocol and reviews it annually.

Any modifications made to the protocol will be reviewed by the IBCSG Ethics Committee and must also be submitted to the appropriate ERB/IRB for information or approval in accordance with local procedures and regulatory requirements and to Health Authorities if required.

Once approved or acknowledged by the appropriate ERB/IRB and by the Health Authorities (if required), the Investigator shall implement the protocol modifications. Protocol modifications for urgent safety matters may be directly implemented following the instructions of IBCSG.

18.2. Regulatory approval procedures

If applicable, in addition to the approval of the Ethics Committee according to national legislation, the protocol, other protocol-related documents including patient information and Informed Consent and other documents as required locally must be submitted to and be approved by the health authority. Documentation of health authority approval must be sent to the IBCSG Data Management Center prior to Participating Center activation.

18.3. Protection of human patients

The IBCSG has an Office for Human Research Protection (OHRP) Federal Wide Assurance (FWA00009439) and follows all of the policies and procedures that are part of that assurance. All potential patients for this trial will receive a full explanation of the trial, its purpose, treatments, risks, benefits, and all of the other items listed in Section 18.4. Additional institution-specific sections should be added to Appendix I as needed.

The medical record must be available for review by the IBCSG audit team and regulatory authorities as described in Section 19.7.

Serious Adverse Event (SAE) Reports are distributed monthly. In addition they are available on the IBCSG website (www.ibcsg.org) for participating Centers.

18.4. Informed Consent

Informed Consent for each patient will be obtained prior to initiating any trial procedures in accordance with the "IBCSG Patient Information Sheet and Informed Consent" (See Appendix I). One signed and dated copy of the Informed Consent must be given to each patient and the original copy must be retained in the Investigator's trial records. The Informed Consent form must be available in the case of data audits. Verification of signed Informed Consent and the date signed are required for randomization to this trial.



The "Declaration of Helsinki" recommends that consent be obtained from each potential patient in biomedical research trials after the aims, methods, anticipated benefits, and potential hazards of the trial, and discomfort it may entail, are explained to the individual by the physician (<http://www.wma.net/en/30publications/10policies/b3/index.html>). The potential patient should also be informed of her right to not participate or to withdraw from the trial at any time. The patient should be told that material from her tumor will be stored and potentially used for additional studies not described in this protocol.

If the patient is in a dependent relationship to the physician or gives consent under duress, the Informed Consent should be obtained by an independent physician. By signing this protocol, the Investigator agrees to conduct the trial in accordance with Good Clinical Practice and the "Declaration of Helsinki."

The IBCSG recognizes that each institution has its own local, national, and international guidelines to follow with regard to Informed Consent. Therefore, we provide a template information sheet and Informed Consent form (Appendix I), which can be downloaded and edited to incorporate information specific to your institution (see www.ibcsg.org). The template Patient Information Sheet and Informed Consent has been written according to ICH guidelines, which state the Informed Consent should adhere to GCP and to the ethical principles that have origin in the "Declaration of Helsinki". The final version should receive the Institutional Review Board/ Local Ethics Committee approval in advance of its use. Centers should send their locally modified PIS/IC to the IBCSG Data Management Center for review and approval before submitting to their Ethics Committee.

18.5. **Premature withdrawal**

18.5.1. Cessation of trial treatment

Patients have the right to refuse further trial treatment at any time during the trial. Patients may also be withdrawn from trial treatment at any time at the discretion of the Investigator due to an adverse event, or based on any other relevant medical condition. Such patients will remain in the trial. The patient will continue to be documented according to protocol.

18.5.2. Withdrawal of consent

Patients have the right to withdraw consent for further trial participation at any time without having to specify the reason. The data recorded up to the time point of withdrawal will continue to be evaluated in the trial. The Investigator should ask the patient for her consent to continue to collect information on her disease and survival status.

It should be documented in both the medical records and in the eCRF (Form 45-COC) whether it is acceptable for the patient to be contacted by telephone for survival status updates. For the patient's safety, an end of treatment visit should be performed and documented in the eCRF.



19. Governance and Administrative Considerations

19.1. Insurance

IBCSG will contract the appropriate liability insurance for this trial. Patients who suffer injuries due to the trial should report them immediately to their physician. The local Center should report all alleged claims immediately to the IBCSG.

19.2. Steering Committee

A Steering Committee will be constituted for this trial. The primary responsibilities of the Steering Committee are twofold. First, the Steering Committee is responsible for maintaining the scientific integrity of the trial, for example, by recommending changes to the protocol in light of emerging clinical or scientific data from other trials. Second, the Steering Committee is responsible for the translation of recommendations of the IBCSG Data and Safety Monitoring Committee into decisions. Membership will include IBCSG officials, trial chair and co-chairs, trial statisticians, representatives from some Participating Centers, and representatives from MSD Pharmaceuticals.

General partition of responsibilities:

The Steering Committee has the authority to make and implement any final decisions, such as sub-studies of the trial or amendments to the trial protocol, and may recommend the termination/early termination of the trial.

The IBCSG Executive Committee is responsible for the implementation of all final decisions taken by the Steering Committee.

The IBCSG Foundation Council decides on the termination/early termination of the trial.

19.3. Data and Safety Monitoring Committee (DSMC)

The trial will be presented for review to the IBCSG Data and Safety Monitoring Committee (DSMC) at each of their semi-annual meetings. Accrual and safety will be monitored. The results of the first stage efficacy analysis will be discussed by the DSMC as soon as available.

19.4. Endpoint Committee

An Endpoint Review Committee will be appointed composed of an immunotherapy expert, the IBCSG medical reviewer and the trial statistician. The committee will review all assessments of response on a monthly basis and in real time. Analysis of the primary endpoint will be based on response data reviewed and confirmed by the Endpoint Review Committee.

19.5. Publication of trial results

IBCSG will publish the results of the trial based on the final study report.



19.6. **Premature discontinuation of the trial**

The trial may be discontinued early in parts or completely if the information on the trial treatment leads to doubt as to the benefit/risk ratio.

The trial can be terminated at any time if the authorization and approval to conduct the Study is withdrawn by ethics committee or regulatory authority decision, insufficient accrual, emerging new data impacting the scientific value of the trial or ethical grounds.

19.7. **Quality Assurance**

The IBCSG conducts trials according to the ICH Good Clinical Practice (GCP) guidelines. The Trial IBCSG Data Manager reviews each CRF. In addition, the IBCSG Medical Reviewer reviews each case at specific timepoints. The IBCSG conducts periodic audit visits to ensure proper trial conduct, verify compliance with GCP, and perform source data verification.

The Investigator should ensure that source documents are made available to appropriately qualified personnel from IBCSG or its designees, or to health authority inspectors after appropriate notification.

MSD-designated staff and quality assurance auditors may audit the conduct of any site and shall be granted reasonable access to facilities during normal business hours. Such auditors shall not be entitled to access, make copies of the source documents and/or to take them away.

At regular intervals during the clinical trial, the Center will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review trial progress, Investigator and patient compliance with clinical trial protocol requirements and any emergent problems. These monitoring visits will include but not be limited to review of the following aspects: patient Informed Consent, patient recruitment and follow-up, SAE documentation and reporting, AEs with pre-specified monitoring documentation and reporting, AE documentation, trial treatment administration, patient compliance with the regimens, drug accountability, concomitant therapy use and quality of data.

19.8. **Protocol adherence**

Investigators ascertain they will apply due diligence to avoid protocol deviations. Under no circumstances should the Investigator contact IBCSG or personnel monitoring the trial to request approval of a protocol deviation, as no deviations are permitted. The Investigator should document and explain any deviations from the approved protocol and promptly report them to IBCSG and to the EC concerned in accordance with the applicable EC policies and procedures. If the Investigator feels a protocol deviation would improve the conduct of the trial this must be considered a protocol amendment, and unless such an amendment is developed and activated by IBCSG and approved by the IRB/IEC/REB it cannot be implemented. All protocol deviations will be documented.



19.9. Data protection

A unique Patient Identification Number (ID) will be assigned by the IBCSG Registration/Randomization System to each patient registered into the trial. The names of the patients will not be disclosed to the IBCSG.

Only the Patient ID will be used to identify a patient on the eCRF. Identification of patients must be guaranteed at the Participating Center. In order to avoid identification errors, Centers should keep a Patient Identification Log containing the patients' name, year of birth, hospital number and the Patient ID allocated by IBCSG.

Regulatory authorities and the pertinent Ethics Committee (ERB/IRB) may have access to patient data on-site. IBCSG audit or monitoring personnel will also have access to such data on-site.

19.10. Record Retention

The Center must retain all essential documents according to ICH GCP. This includes copies of the patient trial records, which are considered as source data, patient Informed Consent statement, laboratory printouts, drug inventory and destruction logs, and all other information collected during the trial. These documents are to be stored until at least 15 years after the termination of the trial. IBCSG guarantees access and availability of the data entered into iDataFAX for at least 15 years after the termination of the trial.

Longer retention may be required for Participating Centers according to national regulations.

In the event that the Principal Investigator retires or changes employment, custody of the records may be transferred to another competent person who will accept responsibility for those records. Written notice of such transfer has to be given to IBCSG and the local Ethics Committee at least one month in advance.

20. Confidentiality

The protocol, CRFs and other protocol-related documents are confidential and are the property of the IBCSG.

21. References

1. Slamon DJ, Leyland-Jones B, Shak S et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344: 783-792.
2. Hurvitz SA, Betting DJ, Stern HM et al. Analysis of Fcγ receptor IIIa and IIa polymorphisms: lack of correlation with outcome in trastuzumab-treated breast cancer patients. *Clin Cancer Res* 2012; 18: 3478-3486.
3. Denkert C, Loibl S, Noske A et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2010; 28: 105-113.
4. Loi S, Sirtaine N, Piette F et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive



- breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol* 2013; 31: 860-867.
5. Mahmoud SM, Paish EC, Powe DG et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *J Clin Oncol* 2011; 29: 1949-1955.
 6. Verma S, Miles D, Gianni L et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 2012; 367: 1783-1791.
 7. Desmedt C, Haibe-Kains B, Wirapati P et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res* 2008; 14: 5158-5165.
 8. Finak G, Bertos N, Pepin F et al. Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 2008; 14: 518-527.
 9. Ignatiadis M, Singhal SK, Desmedt C et al. Gene modules and response to neoadjuvant chemotherapy in breast cancer subtypes: a pooled analysis. *J Clin Oncol* 2012; 30: 1996-2004.
 10. Schmidt M, Bohm D, von Torne C et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. *Cancer Res* 2008; 68: 5405-5413.
 11. Ghebeh H, Mohammed S, Al-Omair A et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. *Neoplasia* 2006; 8: 190-198.
 12. Loi S, Michiels S, Lambrechts D et al. Tumor PIK3CA mutations, lymphocyte infiltration, and recurrence-free survival (RFS) in early breast cancer (BC): Results from the FinHER trial. *J Clin Oncol* 2012; 30.
 13. Park S, Jiang Z, Mortenson ED et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. *Cancer Cell* 2010; 18: 160-170.
 14. Stagg J, Loi S, Divisekera U et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci U S A* 2011; 108: 7142-7147.
 15. Knight DA, Ngiow SF, Li M et al. Host immunity contributes to the anti-melanoma activity of BRAF inhibitors. *J Clin Invest* 2013; 123: 1371-1381.
 16. Balachandran VP, Cavnar MJ, Zeng S et al. Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. *Nat Med* 2011; 17: 1094-1100.
 17. Menard C, Blay JY, Borg C et al. Natural killer cell IFN-gamma levels predict long-term survival with imatinib mesylate therapy in gastrointestinal stromal tumor-bearing patients. *Cancer Res* 2009; 69: 3563-3569.
 18. Zitvogel L, Kroemer G. Anticancer effects of imatinib via immunostimulation. *Nat Med* 2011; 17: 1050-1051.
 19. Rakhra K, Bachireddy P, Zabuawala T et al. CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. *Cancer Cell* 2010; 18: 485-498.
 20. Miao Y, Everly JJ, Gross TG et al. De novo cancers arising in organ transplant recipients are associated with adverse outcomes compared with the general population. *Transplantation* 2009; 87: 1347-1359.
 21. Bidwell BN, Slaney CY, Withana NP et al. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. *Nat Med* 2012.
 22. Chen Q, Zhang XH, Massague J. Macrophage binding to receptor VCAM-1 transmits survival signals in breast cancer cells that invade the lungs. *Cancer Cell* 2011; 20: 538-549.



23. DeNardo DG, Barreto JB, Andreu P et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* 2009; 16: 91-102.
24. Disis ML. Immune regulation of cancer. *J Clin Oncol* 2010; 28: 4531-4538.
25. Brown JA, Dorfman DM, Ma FR et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol* 2003; 170: 1257-1266.
26. Dong H, Strome SE, Salomao DR et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002; 8: 793-800.
27. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 2010; 236: 219-242.
28. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002; 2: 116-126.
29. Thompson RH, Dong H, Lohse CM et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clin Cancer Res* 2007; 13: 1757-1761.
30. Nomi T, Sho M, Akahori T et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin Cancer Res* 2007; 13: 2151-2157.
31. Gao Q, Wang XY, Qiu SJ et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. *Clin Cancer Res* 2009; 15: 971-979.
32. Hamanishi J, Mandai M, Iwasaki M et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci U S A* 2007; 104: 3360-3365.
33. Topalian SL, Hodi FS, Brahmer JR et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; 366: 2443-2454.
34. Hino R, Kabashima K, Kato Y et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. *Cancer* 2010; 116: 1757-1766.
35. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005; 23: 515-548.
36. Okazaki T, Maeda A, Nishimura H et al. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. *Proc Natl Acad Sci U S A* 2001; 98: 13866-13871.
37. Zhang X, Schwartz JC, Guo X et al. Structural and functional analysis of the costimulatory receptor programmed death-1. *Immunity* 2004; 20: 337-347.
38. Chemnitz JM, Parry RV, Nichols KE et al. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J Immunol* 2004; 173: 945-954.
39. Riley JL. PD-1 signaling in primary T cells. *Immunol Rev* 2009; 229: 114-125.
40. Sheppard KA, Fitz LJ, Lee JM et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. *FEBS Lett* 2004; 574: 37-41.
41. Parry RV, Chemnitz JM, Frauwirth KA et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol* 2005; 25: 9543-9553.
42. Agata Y, Kawasaki A, Nishimura H et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol* 1996; 8: 765-772.
43. Vibhakar R, Juan G, Traganos F et al. Activation-induced expression of human programmed death-1 gene in T-lymphocytes. *Exp Cell Res* 1997; 232: 25-28.



44. Nishimura H, Honjo T, Minato N. Facilitation of beta selection and modification of positive selection in the thymus of PD-1-deficient mice. *J Exp Med* 2000; 191: 891-898.
45. Brahmer JR, Tykodi SS, Chow LQ et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; 366: 2455-2465.
46. Nanda R CL, Dees EC. A phase Ib study of MK-3475 in patients with advanced triple-negative breast cancer *Cancer Res* 2015.
47. Emens LA BF, Cassier Ph. Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer (TNBC). *Cancer Res* 2015; 75.
48. Garon EB, Rizvi NA, Hui R et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; 372: 2018-2028.
49. Grosso J HC, Inzunza D. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1; BMS-936558; ONO-4538). *J Clin Oncol* 2013; 31.
50. Taube JM, Klein A, Brahmer JR et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res* 2014; 20: 5064-5074.
51. Weber JS, Kudchadkar RR, Yu B et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. *J Clin Oncol* 2013; 31: 4311-4318.
52. Carbognin L, Pilotto S, Milella M et al. Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. *PLoS One* 2015; 10: e0130142.
53. Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol* 2015; 33: 1974-1982.
54. Okazaki T, Tanaka Y, Nishio R et al. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat Med* 2003; 9: 1477-1483.
55. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989; 10: 1-10.
56. Blackwell KL, Burstein HJ, Storniolo AM et al. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J Clin Oncol* 2010; 28: 1124-1130.
57. Baselga J, Gelmon KA, Verma S et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. *J Clin Oncol* 2010; 28: 1138-1144.
58. Baselga J, Cortes J, Kim SB et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* 2012; 366: 109-119.
59. Pistilli B, Urruticoechea A, Chan S et al. PH IB/II Study of BKM120 plus Trastuzumab (T) in Patients with T-resistant HER2+ Advanced Breast Cancer (BC). Presented at ESMO 2012. *Annals of Oncology* 23 (Supplement 9) 2012; ix116-ix143.
60. Atkinson EN, Brown BW. Confidence limits for probability of response in multistage phase II clinical trials. *Biometrics* 1985; 41: 741-744.
61. Bonetti M, Gelber RD. Patterns of treatment effects in subsets of patients in clinical trials. *Biostatistics* 2004; 5: 465-481.



